# Synthesis of peptide mimetics

Sage, Matthew Arthur

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1995

*Awarding institution:*
University of Bath

# SYNTHESIS OF PEPTIDE MIMETICS

Submitted by

**Matthew Arthur Sage**

for the degree of Ph.D.

of the University of Bath

1995

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## ABSTRACT

*N*-protected α-amino epoxides, derived from α-amino acids were synthesised in a stereoselective manner. From these homochiral precursors several dipeptide analogues were prepared. These include the hydroxymethyl dipeptide, (2*R*,3*S*,2'*S*)-2-hydroxy-1-(*O*-methyl leucinyl)-3-phthaloylaminobutane, synthesised *via* a novel oxirane ring-opening reaction using the Lewis acid $BF_3.OEt_2$ and (L)-leucine-*O*-methyl ester. The hydroxymethyl dipeptide, (2*S*,3*S*,2'*S*)-2-hydroxy-1-(*O*-methyl leucinyl)-3-phthaloylaminobutane, was prepared *via* a *N*-protected α-amino *O*-mono protected diol, which itself was derived from the corresponding α-amino alkene or α-amino epoxide. The chloromethyl dipeptides, (2*R*,3*R*,2'*S*)- and (2*S*,3*S*,2'*S*)-1-chloro-2-(*O*-methyl leucinyl)-3-phthaloylaminobutane, the aziridine dipeptides, (1*R*,2*S*,2'*S*)- and (1*S*,2*R*,2'*S*)-1-(methyl-4'-methylpentanoate)-2-(1-phthaloylaminoethyl) aziridine and the hydroxyethylamines, (2*R**,3*S**,2'*S*) and (2*S**,3*S**,2'*S*)-2-hydroxy-1-(O-methyl leucinyl)-3-phthaloylaminobutane were also prepared from *N*-protected α-amino epoxides.

Molecular modelling studies of the pseudotripeptide Ac-*N*-Ala-[$CH(CH_2OH)NH$]-Leu-NHMe were carried out to determine the potential for β-turns. φ,ψ-Maps were generated to find the lowest energy conformations. Docking of this lowest energy conformer of the potential inhibitor into the active site of human fibroblast collagenase, allowed for determination of the energy of binding.

# ABBREVIATIONS

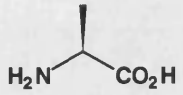
| | |
|---|---|
| AI | angiotensin I |
| AII | angiotensin II |
| AIII | angiotensin III |
| Ac | acetyl |
| ACE | angiotensin converting enzyme |
| 2-adoc | adamantyloxycarbonyl |
| AcOH | acetic acid |
| ADDP | 1,1'-(azodicarbonyl)dipiperidine |
| Ala | alanine |
| amp | 2-(aminomethyl)pyridine |
| amu | atomic mass units |
| aq | aqueous |
| Arg | arginine |
| as | asymmetric stretch |
| Asn | asparagine |
| Asp | aspartic acid |
| Boc | *tert*-butoxycarbonyl |
| Bom | benzyloxymethyl |
| BOP | benzotriazoleoxytris(dimethylamino)phosphonium hexafluorophosphate |
| BMS | borane dimethylsulfide |
| Bn | benzyl |
| BnBr | benzyl bromide |
| Cal | cyclohexylalanine |
| cat. | catalytic |
| Cbz | benzyloxycarbonyl |
| CCK | cholocystokinin |
| C.I. | chemical ionisation |
| C.N.S. | central nervous system |
| CSA | camphorsulfonic acid |
| CTMAC | cetyltrimethylammonium chloride |
| Cys | cystine |
| $\delta$ | deformation |
| DBN | 1,5-diazabicyclo[4.3.0]non-5-ene |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| DCC | 1,3-dicyclohexylcarbodiimide |

| | |
|---|---|
| DCE | 1,2-dichloroethane |
| DCM | dichloromethane |
| d.e. | diastereomeric excess |
| DEAD | diethyl azodicarboxylate |
| DEPC | diethyl pyrocarbonate |
| DEPT | Distortionless Enhancement by Polarisation Transfer |
| DIPEA | diisopropylethylamine |
| DiBAL-H | diisobutylaluminium hydride |
| DMAP | 4-dimethylaminopyridine |
| DMF | dimethylformamide |
| DMPU | 1,3-dimethyl-3,4,5,6-tetrahydro-2[1H]-pyrimidinone |
| DMS | dimethylsulfide |
| DMSO | dimethylsulfoxide |
| DME | dimethoxyethane |
| DNA | deoxyribonucleic acid |
| DNP | dinitrophenylhydrazine |
| DPPA | diphenylphosphoryl azide |
| EDC | 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide |
| e.e. | enatiomeric excess |
| E.I. | electronic ionisation |
| eq | equivalent |
| Et | ethyl |
| EtOAc | ethyl acetate |
| F.A.B. | fast atomic bombardment |
| Fmoc | fluorenylmethoxycarbonyl |
| Ftr | *N*-formyl tryptophan |
| Gln | glutamine |
| Glu | glutamic acid |
| GRF | Growth Hormone-Releasing factor |
| Gly | glycine |
| His | histidine |
| HIV-1 | Human Immunodeficiency Virus type 1 |
| HMPA | hexamethylphosphoramide |
| HOBt | 1-hydroxybenzotriazole |
| HPLC | high pressure liquid chromatograpy |
| hr | hour |
| Hyp | hydroxyproline |
| Iaa | see Xaa |

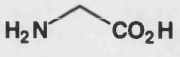
| | |
|---|---|
| $IC_{50}$ | half maximal inhibition |
| Ile | isoleucine |
| Iva | isovalerate |
| KO$^t$Bu | potassium *tert*-butoxide |
| LDA | lithium diisopropylamide |
| lit. | literature |
| Leu | leucine |
| LHRH | Lutinising Hormone-Releasing hormone |
| *m*-CPBA | *meta*-chloroperbenzoic acid |
| Me | methyl |
| MeOH | methanol |
| Met | methionine |
| min | minute |
| m.p. | melting point |
| Ms | methanesulphonyl (mesyl) |
| NaHMDS | sodium bis(trimethylsilyl)amide |
| Nal | naphthylalanine |
| NBS | *N*-bromosuccinimide |
| Nle | norleucine |
| NMO | 4-methylmorpholine *N*-oxide |
| n.m.r. | nuclear magnetic resonance |
| nOesy | nuclear Overhaüser effect spetroscopy |
| Pam | 4-(carboxamidomethyl)benzyl ester |
| Pen | penicillamine |
| PCC | pyridinium chlorochromate |
| PDC | pyridinium dichromate |
| Ph | phenyl |
| Phe | phenylalanine |
| phtN | phthaloylamino |
| Pr | propyl |
| Pro | proline |
| psi | pounds per square inch |
| *p*-TSA | *para*-toluenesulfonic acid |
| RAS | renin-angiotensin system |
| $R_f$ | retention factor |
| RMP | rifampicin |
| RT | room temperature |
| Ser | serine |

| | |
|---|---|
| $S_N1$ | substitution nucleophilic unimolecular |
| $S_N2$ | substitution nucleophilic bimolecular |
| Sta | statine; (3*S*, 4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid |
| TBAF | tetrabutylammonium fluoride |
| TBAI | tetrabutylammonium iodide |
| TBDMS | *tert*-butyldimethylsilane |
| TBDPS | *tert*-butyldiphenylsilane |
| TBP | tributylphospine |
| TcBoc | 2',2',2'-trichloro-1',1'-dimethylethoxycarbonyl |
| TcBoc-ONSu | *N*-(2',2',2'-trichloro-1',1'dimethylethoxycarboxy)succinimide |
| TEBAC | triethylbenzylammonium chloride |
| Tf | trifluoromethanesulfonate (triflic) |
| TFA | trifluoroacetyl |
| TFAA | trifluoroacetic anhydride |
| THF | tetrahydrofuran |
| Thi | thienyl amine |
| Thr | threonine |
| Tic | 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid |
| TPAP | tetrapropylammonium perruthenate (VII) |
| Tr | triphenylmethane (trityl) |
| Trp | tryptophan |
| Ts | *p*-toluenesulphonyl (tosyl) |
| Tyr | tyrosine |
| Val | valine |
| TIP-[ψ] | Tyr-Tic-ψ[$CH_2$NH]-Phe |
| TIPP-[ψ] | Tyr-Tic-ψ[$CH_2$NH]-Phe-Phe |
| T.l.c. | thin layer chromatography |
| TMS | trimethylsilane |
| TMSOTf | trimethylsilyl trifluoromethanesulfonate |
| Xaa | amino acid (also Yaa) |
| Z | see cbz |

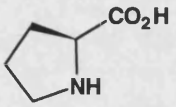
## Naturally occuring Amino Acid abbreviations



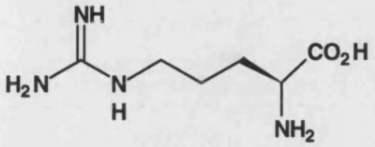
alanine (Ala)



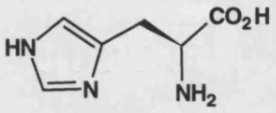
glycine (Gly)



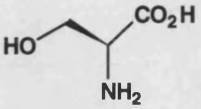
proline (Pro)



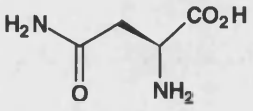
arginine (Arg)



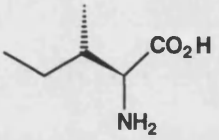
histidine (His)



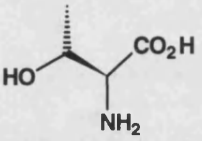
serine (Ser)



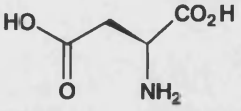
asparagine (Asn)



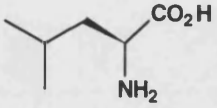
isoleucine (Ile)



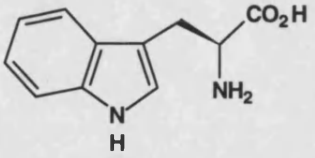
threonine (Thr)



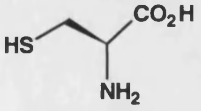
aspartic acid (Asp)



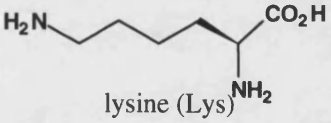
leucine (Leu)



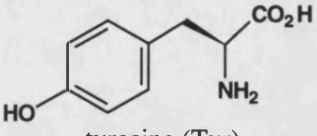
tryptophan (Trp)



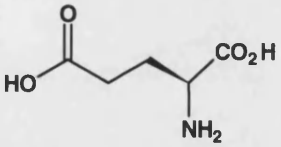
cystine (Cys)



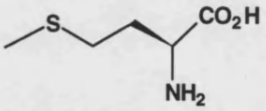
lysine (Lys)



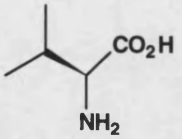
tyrosine (Tyr)



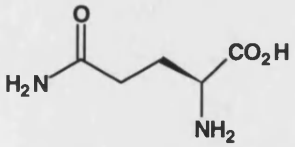
glutamic acid (Glu)



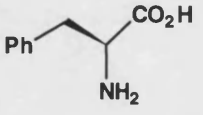
methionine (Met)



valine (Val)

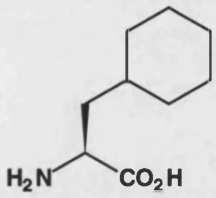


glutamine (Gln)

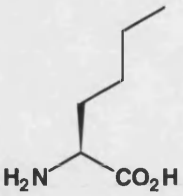


phenylalanine (Phe)

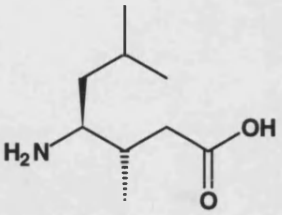
## Novel Amino Acid Abbreviations



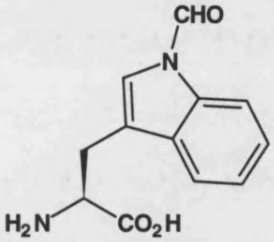

cyclohexylalanine (Cal)



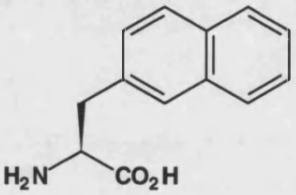

norleucine (Nle)



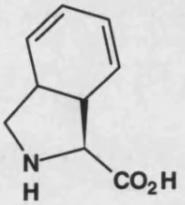

statine (Sta)



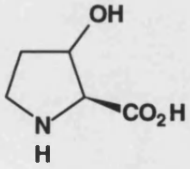

*N*-formyltryptophan (Ftr)



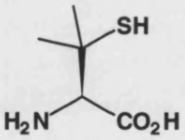

naphthalanine (Nal)




1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic)




hydroxyproline (Hyp)




penicillamine (Pen)

## CONTENTS

# INTRODUCTION

## 1.0 Background

Over the last decade there has been an increased interest in the preparation of transition-state analogues and their incorporation into various sized peptides for conformational and biological purposes. More recently there has been an explosion in the number of peptidomimetic preparations for the treatment of AIDS.

The aim of the project described in this thesis was to establish a new family of peptidomimetics and to examine their potential as transition-state mimetics. Molecular modelling studies would complement binding studies, with the objective of inhibiting collagenase.

## 1.1 Enzyme action[1]

What are enzymes?

*Enzymes* are proteins that catalyse reactions in biological systems. They vary in molecular weights from several thousands to several millions, but can catalyse transformations on molecules as small as carbon dioxide.

When enzymes interact with substrates, to form enzyme-substrate complexes, it is these complexes which catalyse reactions, transforming the substrate into products on release.

Every enzyme has a unique protein structure, defined by a characteristic amino sequence. This is what makes enzymes substrate specific.

There have been many rationalisations put forward to represent the mechanism for enzyme catalysis. Most modern theories originate from that of Haldane[2] in 1930, who introduced the concept that enzyme-substrate complexes require additional activation energy prior to the reaction, which presumably arises from substrate strain on the transition-state geometry. Eyning[3] in 1935, developed the idea of transition-state theory, which was the basis for Pauling's[4] hypothesis that enzymes are flexible templates, designed by evolution to be complementary to the structure of the substrate at the transition-state of the reaction, rather than at the ground state. As the reaction proceeds towards the transition-state, the enzyme interacts more effectively (increased binding energy) with the transition-state geometry and electronic environment, thus accelerating the reaction. This was remarkably accurate considering how little there was known about enzymes at the time. Indeed, it is possible to make some generalisations concerning the methods by which enzymes catalyse reactions:-

i. Deforming a configuration of atoms away from their equilibrium position revises the energy of the system. If an enzyme can stretch a bond, the energy of the molecule will become closer to the transition-state and thus bond cleavage will be aided.

ii. Enzymes which can concentrate and orientate reactants will catalyse reactions. Simply bringing together the reactants can theoretically cause an acceleration, largely due to the change in concentration caused, and

iii. Nucleophilic attack with an electrophile can be accelerated by making the electrophile more electrophilic and *vice-versa*. This is electronic activation.

Enzymes bind substrates at a region of the enzyme termed the active site. Enzymes are very large with the atoms in the protein being closely packed. The entire protein outside the active site may be functioning to hold the active site in the proper geometry for catalysis. Another aspect of enzyme catalysis, may involve channelling the substrate into the active site. Once binding sites link to specific groups in the substrate, relative orientation is fixed, so that reacting functionalities are in the vicinity of the catalytic domain of the enzyme.

The binding specificity can be absolute *i.e.* essentially only one substrate forms an enzyme-substrate complex with a particular enzyme, thus leading to one product, or the binding specificity may be very broad, in which case many molecules of related structure can be converted to products. As enzymes are chiral (mammalian enzymes are made up of only L-amino acids encoded from DNA) the binding energy of the enzyme-substrate complex (E-S) for one enantiomer may be much higher than the other enantiomer, due to differential binding interactions caused by steric effects. If this energy difference is very large, only one E-S complex may form. Alternatively, both E-S complexes may form, but only one may lead to product formation. If the E-

S complex is formed, but not converted to the product, it is said to undergo "non productive binding" to the enzyme.

## 1.2 Enzyme Inhibition and Inactivation

Many diseases, or the symptoms of diseases, are caused by an imbalance of a metabolite in the body, either from an infestation by a foreign body or from abnormal cell function. If this imbalance can be normalised and the foreign organism and abnormal cells can be destroyed, then the symptoms can be remedied. Many of these situations can be effected by specific enzyme inhibition.

If a compound can slow down or block enzyme catalysis it is said to be an *enzyme inhibitor*. If the interaction is irreversible (in which case usually covalent bonding is involved), then the compound is referred to as an *enzyme inactivator*. Many drugs function as enzyme inhibitors or inactivators.

If for example, a cell has a deficiency of the substrate for an enzyme, and as a result a disease state develops, then inhibition of the enzyme will reduce the degradation of the substrate, thus returning the cell to its normal state.

An ideal enzyme inhibitor should be totally specific for the one target enzyme. Since this is rare, highly selective inhibition is a more realistic objective. Unfortunately, when treating various organisms and tumour cells the enzymes that are essential for their growth are also vital to human health. Although this is a problem, many inhibitors have been developed as the tumour cells replicate at a much faster rate than do most normal human cells, and foreign organisms contain many enzymes that are non-essential for human health or not present in humans. The use of penicillins and inhibition of the shikimic acid pathway are two examples which are non toxic to humans.

There are several forms of enzyme inhibition: competitive, uncompetitive, non-competitive, as well as mixed, partial and allosteric.

Competitive reversible inhibitors often have structures similar to those of the substrates or products of the target enzymes, and which bind at the substrate binding sites, thereby blocking substrate binding. The enzyme bound inhibitor either lacks the appropriate reactive group or is held in an unsuitable orientation with respect to the catalytic site. Interaction of the inhibitor with an enzyme can occur at a site other than the substrate binding site and still result in the shutdown of the catalytic conversion of the substrate to products. This process involves an inhibitor-induced conformational change of the enzyme, giving a form of the enzyme that is unable to bind to the substrate properly, this form of inhibitor is known as a *non-competitive reversible inhibitor.*

Irreversible inhibitors, also known as *active site-directed irreversible inhibitors* or *enzyme inactivators*, are compounds whose structures are similar to those of the substrate or product of the target enzyme. Irreversible inhibitors generally bind to the active site forming a covalent bond, this may prevent the substrate binding or it may destroy some component of the catalytic site. Unlike reversible inhibitors, once the irreversible inhibitor binds with the target enzyme, the complex cannot dissociate and therefore the enzyme remains inactive. This may mean smaller and fewer doses of the drug are required. Although the enzyme has been inactivated, this does not mean one dosage will remedy the disease, because our bodies are constantly encoding more proteins, so as the enzyme loses activity, other copies are synthesised, but this process is rather slow, and can take hours to several days.

Any data discussed in the text will be associated with enzyme inhibition *in vivo* by various compounds synthesised and their activities will be represented in terms of

binding affinities, $K_i$ or $IC_{50}$ values, where $IC_{50}$ is the concentration required to produce half maximal enzyme inhibition.

### 1.2.1 Peptide mimetics as enzyme inhibitors

A peptide mimetic is a molecule that mimics the biological activity of a peptide, but is no longer peptidic in chemical nature. This term is sometimes used to describe molecules that are no longer completely peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids. Strictly speaking, peptide mimetics are molecules that no longer contain any peptide bonds (i.e. amide bonds between amino acids) and have a molecular weight less than 700 Da.

## 1.3 Design of peptide mimetics

There are essentially two ways of discovering peptide mimetics: rational design and random screening. Random screening has provided the majority of presently known peptide mimetics, however, it is costly, labour intensive and does not always give results. On the other hand, rational design is relatively inexpensive. Ideally a combination of both random screening and rational is desirable.

Moore[5] has discussed the three essential steps of rational design. The first step is to identify pharmocophoric groups that are responsible for enzyme inhibition. Using peptide libraries the structure-activity relationships of a given peptide can be acquired to identify the crucial pharmocophoric groups, together with spectroscopic data, which can provide further information regarding potential pharmocophores.

Step two involves modelling, minimisation and molecular mechanics, using the spatial arrangement of known pharmocophores acquired from n.m.r. spectroscopy or X-ray crystallography. Following minimisation and molecular dynamics simulation

the conformational model established can be used to consider possible receptor mechanisms. This can be useful as the dynamics of folding of peptides in the receptor environment often produces a particular characteristic in the peptide which the receptor "reads".

The final step is the mimetic design. It is now important to probe the spatial relationship of the pharmocophores and then guess what may happen at the receptor site.

The process depends on selecting a template and then mounting the pharmocophores on it. The simplest design is a binary mimetic in which two pharmocophores are mounted, becoming more difficult the more you mount, with four being the limiting situation.

The amide bond is the focal point when it comes to enzymatic stability of a peptidic substance. It can function simply as a spacer, connecting two pharmocophoric groups, it can provide specific spatial orientation and can ultimately be involved in binding of the peptide to the enzyme through hydrogen bonding. A general strategy in drug development, is to establish the cleavage site for a proteolytic process, and replace this unit with an amide isostere. A huge number of isostere replacements have been developed that resist proteolytic degradation, when incorporated into peptide mimetics. This will be discussed later.

There are clear advantages to using peptide mimetics as opposed to the native peptides, which commonly exhibit poor bioavailability and short duration of action resulting from enzymic degradation. Peptide mimetics offer greater bioavailability as they are small, and have longer duration of action. Finally, peptides suffer from problems associated with stability, storage and immunoreactivity, whereas these problems may not be experienced with peptide mimetics.

Analogues of natural peptide substrates have been prepared in which conformational restraints and non-peptide linkages have been introduced, as well as by changing the natural amino acid sequence and making amino acid side chain substitutions.

The course of preparing these peptide mimetics have been based on solid phase and solvent phase synthetic chemistry. More recently, there has been a move towards molecular biology, which has now become the driving force behind screening and the establishment of macromolecular targets. However, much of the research has been directed at peptide and protein therapeutics which still display the disadvantages of peptides. Because of those limitations, there is still a lot of reliance on non-peptide units. Small molecules are likely to remain the most viable avenue.

### 1.3.1 Peptide Conformation

The geometry of the peptide backbone is shown in Figure 1.[6]

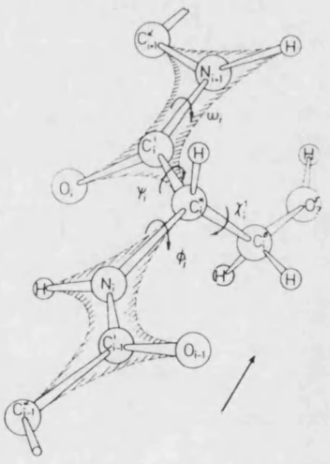
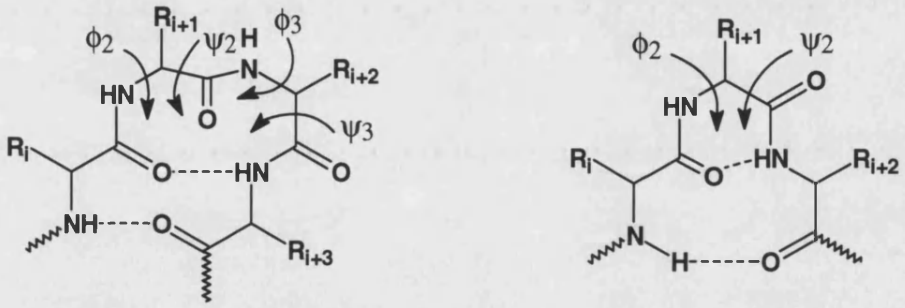


**Figure 1**

In the polypeptide backbone it is generally found that each amide bond adopts a *trans* configuration. The principle torsion angle describing the rotation about N-$C^{\alpha}$ is denoted by $\phi$, that describing rotation about $C^{\alpha}$-C is defined by $\psi$, and that describing rotation about C-N is denoted by $\omega$. The symbols $\phi_i$,$\psi_i$ and $\omega_i$ are used to denote torsion of bonds within the *i*th residue in the case of $\phi$ and $\psi$ and between the *i*th and (*i*+1)th residues in the case of $\omega$; specifically, $\phi_i$ refers to the torsion angle of the

sequence of atoms $C_{i-1}$, $N_i$, $C^{\alpha}_i$, $C_i$; $\psi_i$ to the sequence $N_i$, $C^{\alpha}_i$, $C_i$ $N_{i+1}$; and $\omega_i$ to the sequence $C^{\alpha}_i$, $C_i$ $N_{i+1}$,$C^{\alpha}_{i+1}$; (**Figure 1**)

Both β-turns and γ-turns are well known secondary structures in peptides and proteins, and cause a reversal of a peptide chain. These turns may or may not be stabilised by an intra-turn hydrogen bond. In the case of the β-turn it may occur between the carbonyl of residue *i* and the NH of residue *i*+3, while in the γ-turn it may occur between the carbonyl of residue *i* and the NH of residue *i*+2.




β-turn γ-turn

**Figure 1**

Conformationally, a β-turn is fully defined by the (φ,ψ) torsions of the middle two residues: ($\phi_2$,$\psi_2$) and ($\phi_3$,$\psi_3$); and for the γ-turn only the single torsion ($\phi_2$,$\psi_2$) is necessary for definition. **Table 1**, shows the types I-III β-turns and their mirror images, and the normal and inverse γ-turns.

**Table 1**

| Turn | $\phi_2$ | $\psi_2$ | $\phi_3$ | $\psi_3$ |
|---|---|---|---|---|
| β-turns type | | | | |
| I | -60 | -30 | -90 | 0 |
| II | -60 | 120 | 80 | 0 |
| III | -60 | -30 | -60 | -30 |
| I' | 60 | 30 | 90 | 0 |
| II' | 60 | 120 | 80 | 0 |
| III' | 60 | 30 | 60 | 30 |
| γ-turns type | | | | |
| turn | 70 to 85 | | 60 to -70 | |
| inverse turn | -75 to -85 | | 60 to 70 | |

φ, ψ values in (°) at position 2 and 3

It is notable that type I and III β-turns are very similar; and they occupy similar regions of ϕ,ψ space and are not distinct types.[7] However, type III can be conveniently placed into a separate category, since it describes a helical turn and may form part of a larger repeating structure.

### 1.3.2 Amide Bond Modifications

Modification of the peptide backbone can increase the biological half-life of a compound compared to its parent compound and also introduce conformational restraints. The tactic of replacing the amide bond with a suitable surrogate or isostere is of particular importance in developing new enzyme inhibitors. Several isosteric amide bond mimetics have been introduced into biologically active peptides *e.g.* ψ [$CH_2S$], ψ[$CH_2NH$], ψ[$SO_2$], ψ[P=O(OH)], ψ[NHCO], ψ[$COCH_2$], ψ[$CH(OH)CH_2$] and ψ[(*E*) or (*Z*) CH=CH] which have all been extensively reviewed.[8,9] These modifications have also been used in numerous enzyme inhibitors *e.g.* matrixmetalloprotease, angiotensin converting enzyme, renin,[8g] aspartic protease and HIV protease[9] and many others.

### 1.3.3 Transition-State Theory

As discussed earlier, Pauling[4] in 1948 developed the transition-state theory. He concluded that an enzyme accelerates the rate of a reaction by stabilising the transition-state, which lowers the free energy of activation. This rate enhancement is achieved by the enzyme altering its conformation, so that it can bind more tightly to the substrate at the transition-state of the reaction. Some enzymes can also apply strain to the substrate and thus distort the complex towards the transition-state of the reaction.

Bernhard and Orgel[10] theorised that transition-state analogues would bind more tightly to an enzyme than the substrate. Thus, a potent enzyme inhibitor would be a stable compound that resembles the substrate at the transition-state of the reaction and not the ground state. A compound that binds more tightly to the enzyme is called a *transition-state analogue inhibitor.*

For one-substrate reactions, the unimolecular conversion of a reactant, S, to a product, P, will occur *via* the reaction pathway with the lowest energy barrier and requires that the reactants have sufficient energy to overcome this barrier (dashed curve, Figure 2). The structure of highest energy, on the lowest energy pathway, is called the transition-state, $S^{\neq}$. This is in equilibrium with the reactants (Equation 1).

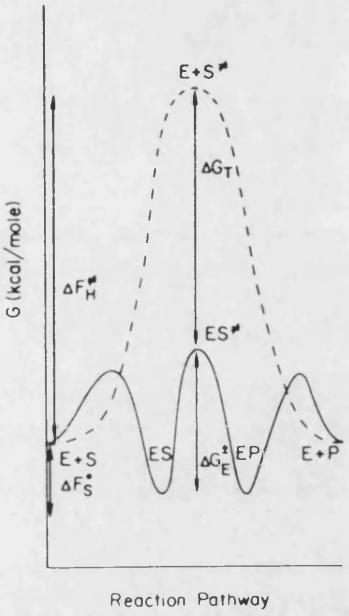
$$K^{\neq} = [S^{\neq}]/[S] \qquad \text{Equation 1}$$

where $K^{\neq}$ is the equilibrium constant for the formation of the transition-state. The rate of reaction is proportional to the concentration of the transition-state, and the proportionality constant is given as (Equation 2).

$$d[P]/dt = kt/h^{*}[S^{\neq}] = kTK^{\neq}/h^{*}[S] = k_{X}{}^{*}[S] \qquad \text{Equation 2}$$

where k is the Boltzmann's constant, h is Plank's constant and T is the absolute temperature.

Consequently, $K^{\neq}$ is equal to the measurable first-order rate constant for the reaction, $k_X$, times the factor h/kT (Equation 2). The difference in the free energy between the reactant and transition-state, $\Delta F^{\neq}_{H}$, is related to the equilibrium constant, $K^{\neq}$ by the thermodynamic equation, $\Delta F^{\neq} = -RT\ln K^{\neq}$, and thus, is also calculable from $k_X$.

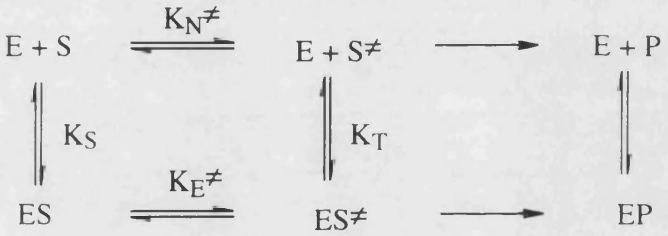



Reaction Pathway, Binding Components

**Figure 2.** (A) Schematic representation of reaction pathway profile for nonenzymatic (---) and enzyme catalyzed hydrolysis of amide bond (-).

**Figure 2**

Application of the transition-state theory to a single-substrate enzymatic reaction and to the corresponding non-enzymatic reaction is shown below.



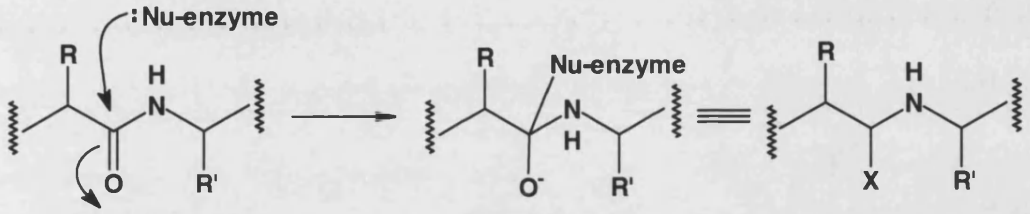

Where $K_S$ is the equilibrium constant for the association of the enzyme, E, $K_N^{\neq}$ and $K_E^{\neq}$ are equilibrium constants for the formation of the transition-state of the non-enzymatic and enzymatic reactions, $S^{\neq}$ and $ES^{\neq}$, respectively, $K_T$ is the equilibrium constant for the binding of $S^{\neq}$ to E to form $ES^{\neq}$. The expression of these equilibrium constants are related by Equation 3.

$$K_T/K_S = K_E^{\neq}/K_N^{\neq} = K_E/K_N \quad \text{Equation 3.}$$

According to the simplest transition-state theory, $K_E^{\neq}$ is related to $K_E$, the first order rate constant for the conversion of ES to EP by the same factor (h/kT) that relates to $K_N^{\neq}$ to $K_N$, the first order rate constant for the corresponding non-enzymatic reaction. This concludes that for reactions that have been reported, that the value for enzymatic

catalysis, expressed by the ratios $K_E/K_N$, for a typical enzymatic reaction, will fall in the range $10^8$ to $10^{14}$. Since the association constant, $K_S$ is usually of the order $10^3$ to $10^5$ $M^{-1}$, then the expected values for $K_T$ are extremely large $10^{15}$ $M^{-1}$. These relationships are shown in Figure 2, in terms of the free energy-reaction pathway profile for a hypothetical single-substrate enzymatic reaction (unbroken line) and the non-enzymatic reaction (broken line).

The action of an enzyme, when hydrolysing the amide linkage of a peptide proceeds *via* nucleophilic attack on the carbonyl to generate a $sp^3$-tetrahedral intermediate.

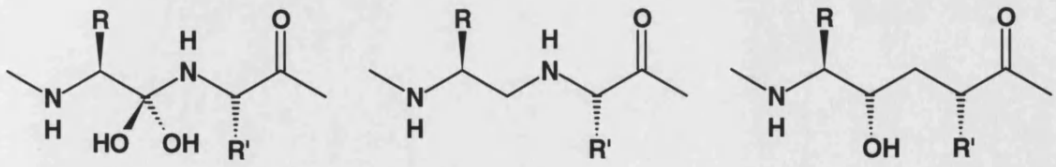



native peptide bond | transition-state (enzyme-substrate complex) | transition-state mimetic

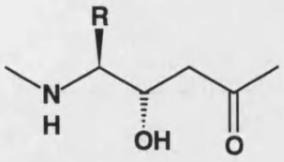
As mentioned earlier, modification of the cleavage site to mimic the $sp^3$-tetrahedral species has resulted in the generation of analogues with high inhibitory potency and has been one of the most widely used strategies in peptide mimetic design.

### 1.4 Reduced Peptide Isosteres

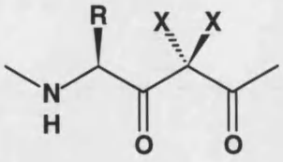
This discussion will be a review of the synthetic and biological chemistry of certain transition-state analogues. This review will concentrate on the reduced amide, hydroxyethylene and hydroxyethylamino isosteres, see Chart 1.



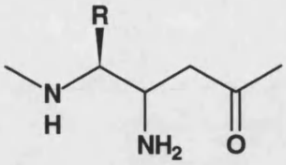

tetrahedral intermediate from water attack | reduced peptide | hydroxyethylene isostere



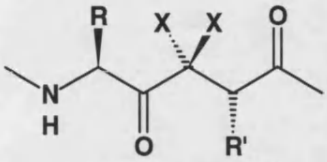
statine
$R=CH_2CHMe_2$



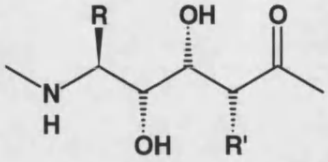
X= H, statone analogue
F, difluoro statone



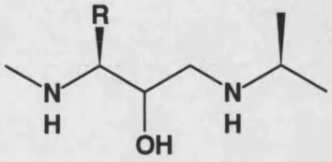
amino statine



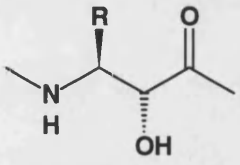
X= H, ketone isostere
F, difluoroketone isostere



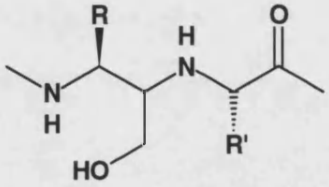
dihydroxy isostere



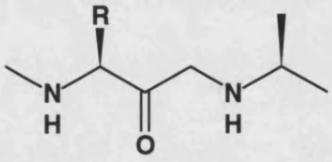
hydroxyethylamine



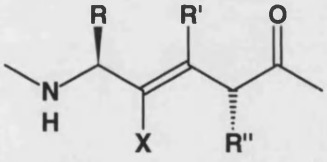
norstatine



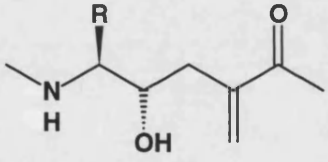
hydroxymethylmethylamine



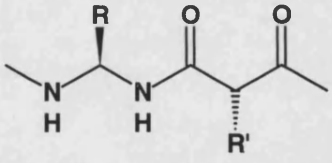
ketomethyl isostere



X= H, double bond isostere
F, fluoroalkene isostere



dehydrohydroxy ethylene isostere



retro-inverso isostere

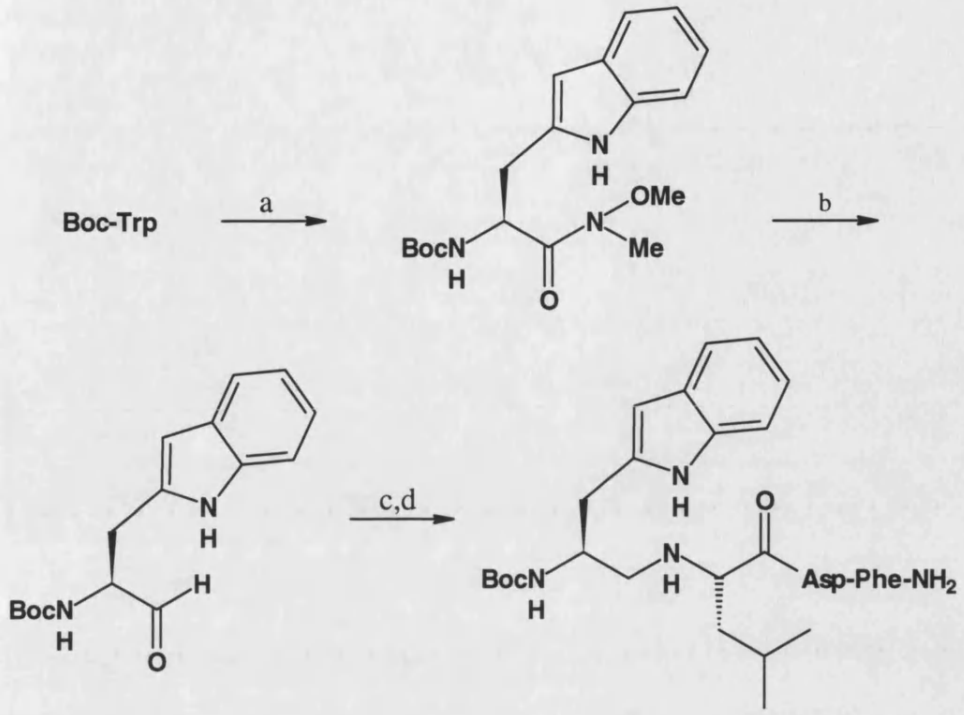
**Chart 1**

### 1.4.1 Reduced Amide Isosteres

Using the transition-state theory, Szelke[11] and co-workers developed potent inhibitors of angiotensin converting enzyme (ACE) and renin which used the reduced peptide isostere $\psi[CH_2NH]$ at the scissle site. Although the isostere contained the tetrahedral geometry of the $sp^3$-transition-state intermediate, it lacked the hydroxyl group. The first potent renin inhibitor was the reduced peptide analogue of the Kokubu's tetrapeptide (Leu-Leu-Val-Phe-OMe), reported by Szelke[12] in 1972, Leu-$\psi[CH_2NH]$-

Leu-Val-Phe-OMe. Although this had moderate potency, a dramatic improvement was achieved with a reduced peptide isostere of Shegg's octapeptide, His-Pro-Phe-His-Leu-ψ[$CH_2NH$]-Leu-Val-Tyr.[13] When the *N*-terminal sequence of the human substrate became known other more potent reduced peptide isosteres were made which incorporated the human cleavage site His-Pro-Phe-His-Leu-ψ[$CH_2NH$]-Val-Val-Tyr, and the human substrate sequence His-Pro-Phe-His-Leu-ψ[$CH_2NH$]-Val-Ile-His, (see review by Greenlee[8g]). Szelke[14] improved the renin inhibitors further by synthesising the reduced peptide analogue Pro-His-Pro-Phe-His-Leu-ψ[$CH_2NH$]-Val-Ile-His-Lys.

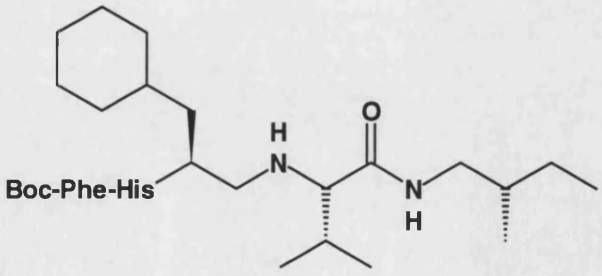
In 1985 Martinez *et al*[15] prepared reduced peptide isosteres of Gastrin, a 17 amino acid hormone isolated from hog antral mucosa, which plays a major role in the stimulation of gastric acid secretion. It was recognised that only the C-terminus, Trp-Met-Asp-Phe-$NH_2$ was responsible for a remarkable range of physiological effects. Martinez *et al*[15] prepared reduced peptide isosteres between each amino acid in this tetrapeptide.[16] Replacement of Trp-Met peptide bond with ψ[$CH_2NH$] gave a compound which stimulated gastric secretion *in vivo*, however Met-Asp peptide bond replacement gave no such release. In 1987 they found that n.m.r. analysis of bond replacements of each peptide link of Boc-Trp-Leu-Asp-Phe-$NH_2$ revealed that the conformational effect of each modification was localised around each of the ψ[$CH_2NH$] substitutions.[17]




a. HCl.N(OMe)Me, BOP or DCC/DMAP; b. $LiAlH_4$; c. Leu-(Bn)Asp-Phe-$NH_2$, $NaCNBH_3$, MeOH, AcOH; and d. 10% Pd/C, $H_2$.

**Scheme 1**

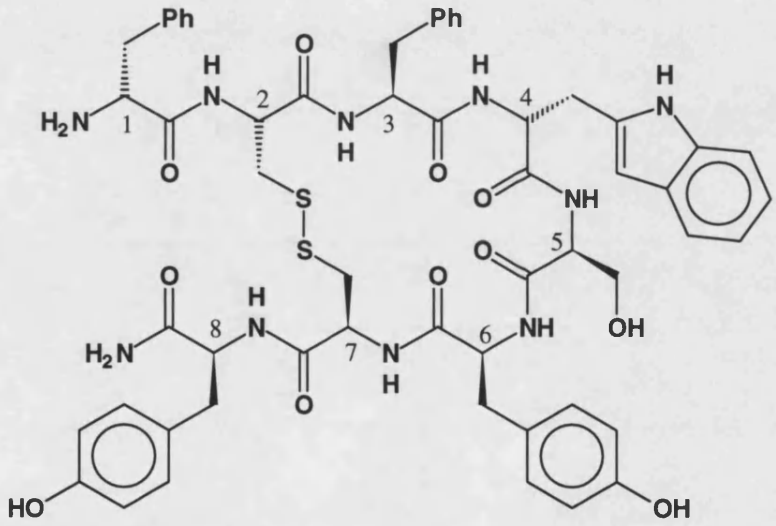
Plattner *et al*[18] developed potent human renin inhibitors, of which the reduced peptide isostere **(1)** was the most active, ($IC_{50}$ 7.8 nM), using the established reductive alkylation conditions employed by Martinez *et al.*[15]




**(1)**

In 1986 van der Elst *et al*[19] prepared reduced peptide analogues of Pro-Leu-Gly-$NH_2$ the C.N.S. active hormone, in the same manner as Martinez *et al*,[15] and showed that

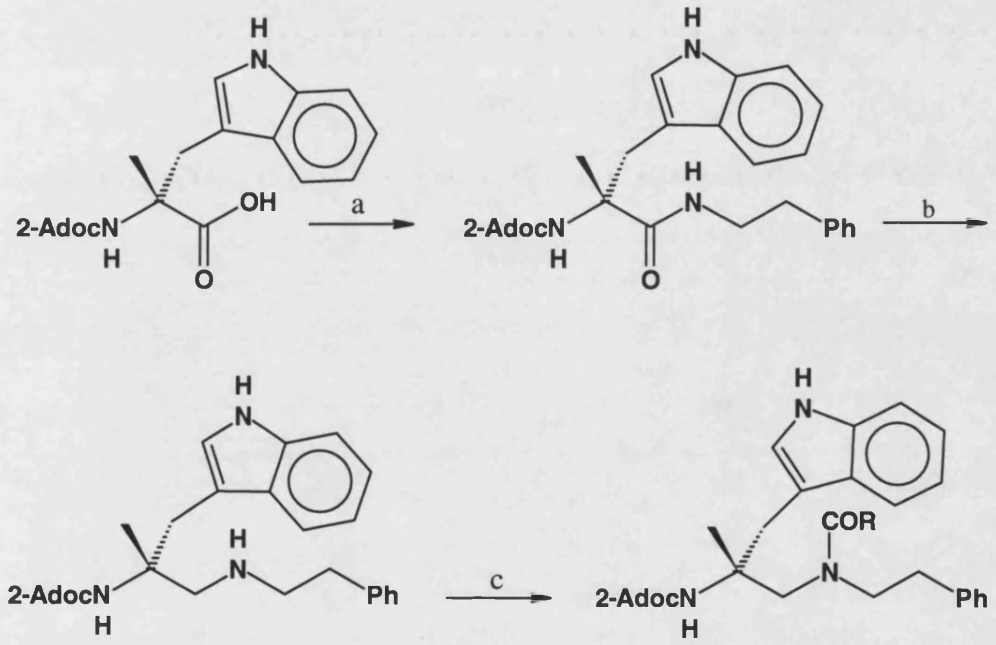
the β-turn conformations were similar to those in the parent hormone, which had similar biological activity. The same group[20] showed that incorporation of ψ [$CH_2NH$] moiety between positions 8 and 9 in deamino-oxytocin and deamino-oxypressin gave analogues with much lower biological activities. Coy *et al*[21] developed a solid phase method for the direct introduction of ψ[$CH_2NH$], utilising a cyanoborohydride reductive amination of a resin-bound peptide amine with a Boc-amino aldehyde. This enabled them to synthesis a series of ψ[$CH_2NH$] pseudopeptide analogues of the highly potent somatostatin octapeptide (**2**). They found sequential modifications of $Cys^2$ and $Cys^7$ carbonyls provided analogues with the highest activities for inhibition of the release of growth hormone.




**(2)**

Coy *et al*[22] also used this methodology to develop analogues of the Growth Hormone Releasing-Factor (1-29) Amide. They investigated the effects of sequential peptide bond replacement by the isostere ψ[$CH_2NH$] in the active region [GRF(1-29)$NH_2$], and found weak agonists and one antagonist. Coy *et al*[23] also developed Substance-P receptor antagonists and prepared reduced peptide bond pseudopeptide analogues of secretin.[24]

In the same year, Martinez *et al*[25], developed reduced amide isosteres of all the peptide bonds in the C-terminal heptapeptide of cholecystokinin (CCK), Z-Tyr($SO_3^-$)-Nle-Gly-Try-Nle-Asp-Phe-$NH_2$. They found that the peptide bonds were not crucial for pancreozymin activity or for binding to CCK receptors, as all the modified peptides stimulated amylase secretin. Later, Rees *et al*[25b] incorporated a reduced peptide bond surrogate in a CCK-B receptor ligand. The synthetic pathway for the preparation of these surrogates are shown in **Scheme 2**.

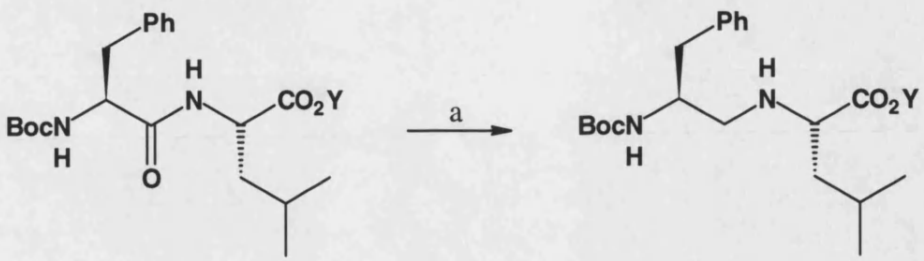



a. $PhCH_2CH_2NH_2$, DCC, EtOAc; b. $LiBH_4$, TMS; and c. RCOCl, $Et_3N$, R=alkyl.

**Scheme 2**

They also prepared analogues using standard reductive amination.

Oyamada and Ueli[26] prepared the modified analogue [Phe-ψ($CH_2NH$)-Leu$^{4-5}$] leucine-enkaphalinin in good yield *via* the reduction of the amide bond by borane (**Scheme 3**).




Y = $CH_2CH_2OMe$, Me or $^tBu$

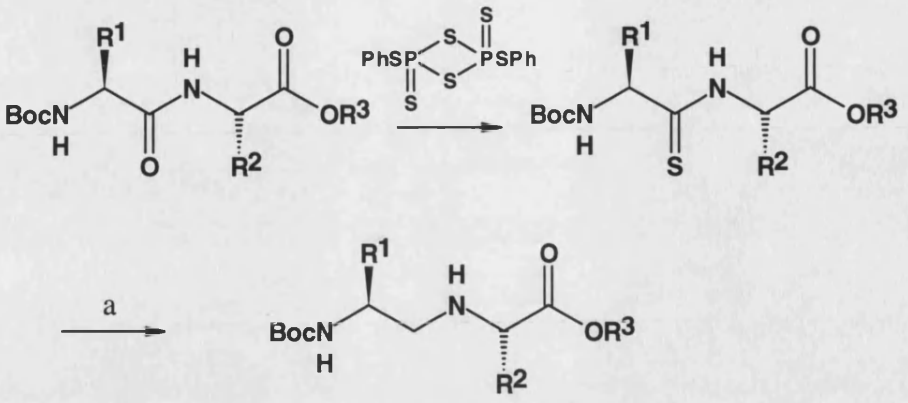
a. $BH_3$-THF, THF.

**Scheme 3**

Sawyer *et al*[27] prepared several potent renin inhibitors incorporating the reduced amide ψ[$CH_2NH$], the most potent being the analogue Ac-Ftr-Pro-Phe-(*N*-MeHis)-Phe-ψ[$CH_2NH$]-Phe-$NH_2$, (Ftr = *N*-formyl tryptophan). These were synthesised using solid phase reductive amination as described by Coy *et al.*[21] This same methodology was used by Coy *et al*[28] to prepare reduced amide analogues of Lutenising Hormone-Releasing Hormone (LHRH).

Coy *et al*[29] also investigated the effect reduced amide isosteres had on bombesin a potent autocrine growth factor in human small cell lung carcinoma systems. Bombesin (*p*-Glu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-$NH_2$) when modifi-ed at $Val^{10}$-$Gly^{11}$ this gave a 30% increase in potency over the parent peptide. The modified ($Leu^{13}$-ψ[$CH_2NH$]-$Met^{14}$) bombesin exhibited a 100-fold improvement in binding affinity compared to previous reported bombesin receptor antagonists.

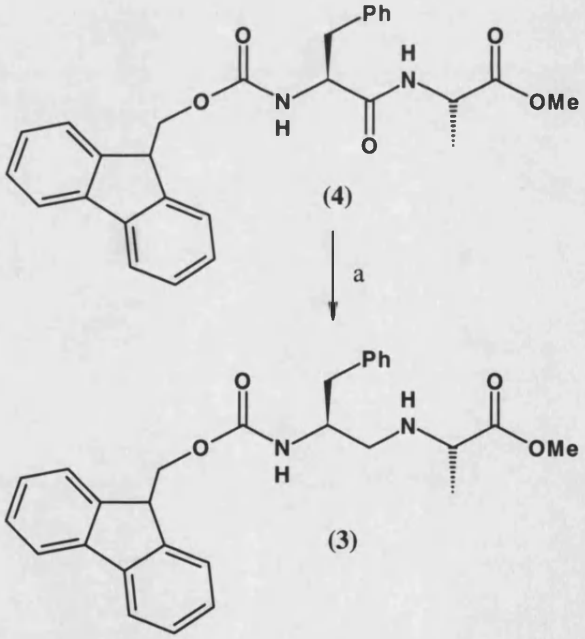
Guziec and Wasmund[30] and Geyer *et al*[48] developed a completely new method for the preparation of reduced amide isosteres, using Raney nickel desulfurisation, (**Scheme 4**).




a. i. RaNi, ii. 1) $Et_3O^+BF^-_4$, 2) $NaBH_4$ or iii. Ni-Boride.

**Scheme 4**

Gianmis and Sandhoff[31] developed a novel way of preparing reduced amide isosteres, using metal borohydrides. They successfully prepared the reduced amide isostere **(3)** using $LiBH_4$ and TMS in moderate yield from the corresponding dipeptide **(4)**, **Figure 3**.




a. i. $LiBH_4$, TMS, THF, 60% or ii. $NaBH_4$, TMS, THF, 55%.

**Figure 3**

Van Binst *et al*[32] synthesised bradykinin analogues Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg with reduced peptides between $Gly^4$-$Phe^5$, $Phe^5$-$Ser^6$ and $Pro^7$-Phe.[8] Some

displayed high potency and prolonged activity. More recently Straub *et al*[33] prepared the reduced peptide analogue RMP-7 {Arg-Pro-Hyp-Gly-Thi-Ser-Pro-(4-Me-Tyr)-ψ[$CH_2NH$]-Arg-OH}, Thi = thienyl amine. The reduced amide was prepared using the procedure of Coy and Sasaki,[34] and also Ho *et al.*[35]

Martinez *et al*[36] prepared reduced bond analogues of C-terminal hexapeptide of neurotensin, Arg-Arg-Pro-Tyr-Ile-Leu-OH. They sequentially replaced each peptide bond with the reduced bond ψ[$CH_2NH$]. Replacement of $Lys^8$-$Lys^9$ with ψ[$CH_2NH$] produced an analogue which had the same affinity for neurotensin receptors, but was 10 times more potent in stimulating guinea pig ileum contraction. *N*-terminal protection (Boc group) decreased potency compared to the free amine.

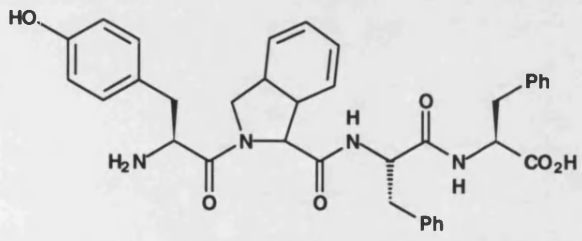
Christos *et al*[37] also prepared neurotensin C-terminal modified pentapeptides, using the standard reductive amination with cyanoborohydride. They found that the reduced amide pseudopeptide did show analgesic activity.

Both Michelot *et al*[38] and Harbeson *et al*[39] prepared neurokinin pseudopeptide analogues, with reduced amide bonds. They showed little improvement on the native peptide. These analogues were prepared using standard reductive alkylation techniques.

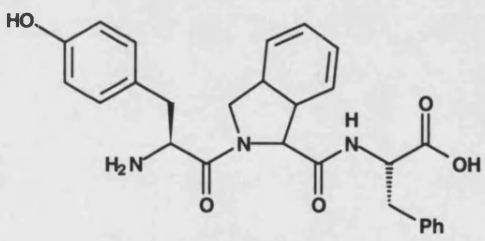
Van Binst *et al*[40] prepared morphiceptin and β-casomorphin-5 analogues which contained reduced peptide bonds. They prepared these analogues using a combination of solid phase methodology and reductive amination. They noted that there was slow *cis/trans* isomerisation between the amino acid and proline bonds in the sequences. All analogues showed enhanced binding affinity for the μ-receptor, and very low binding to the δ-receptor compared to D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-$NH_2$, (Pen = penicillamine).

Reduced amide analogues have also been used to examine the importance of the back-bone structure and flexibility of ligand interactions with insulin. Tager *et al*[41] prepared the reduced amide analogues using solid-phase, solution-phase and semi-synthetic methods. They found that the reduced amide analogues retained the ability to form organised metal ion-co-ordinated complexes ($Co^{2+}$) in solution, but reduction of the peptide bonds proximal and distal to the critical side chain of $Phe^{B25}$ produced analogues which had reduced binding potency.

Schiller *et al*[42] developed a new class of opioid peptide derived δ-antagonists which contained the 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic). The two prototype antagonists were the tetrapeptide Tyr-Tic-Phe-Phe **(5)** and the tripeptide Tyr-Tic-Phe **(6)**, **Figure 4**. They prepared reduced amide isosteres of these two antagonists, with the reduced bond between Tic-Phe. These were synthesised using standard reductive amination.




TIPP (**5**)



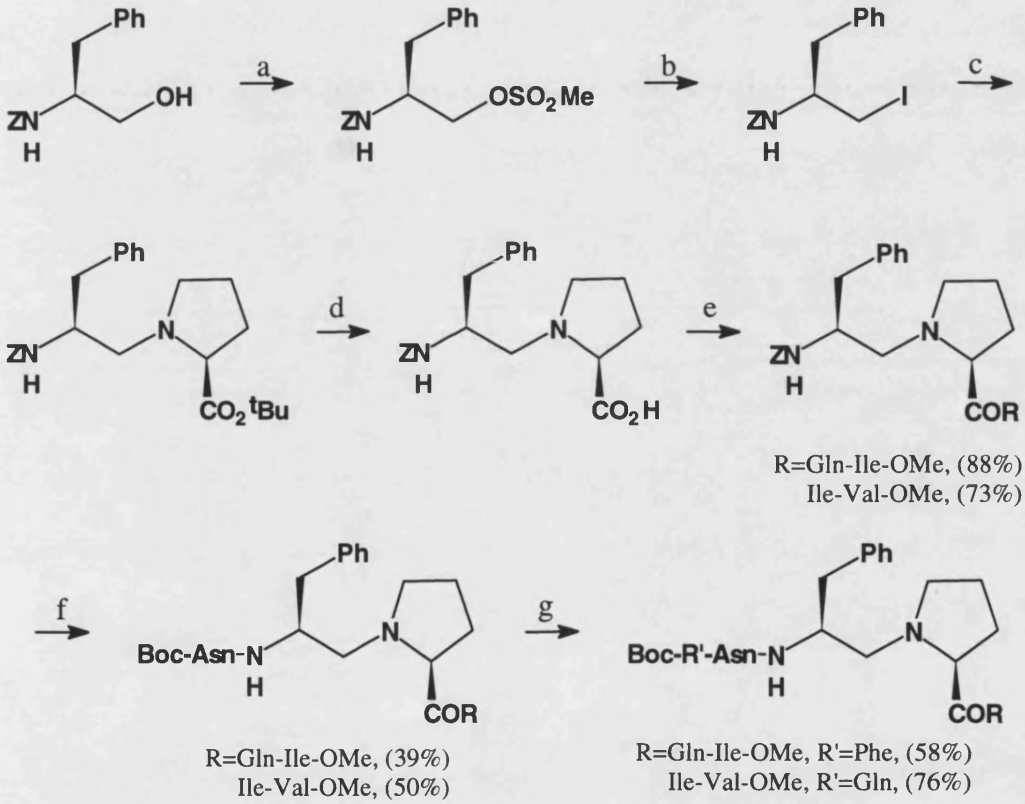

TIP (**6**)

**Figure 4**

The analogue Tyr-Tic-ψ[$CH_2NH$]-Phe-Phe (TIPP[ψ]) was the more potent compound and displayed subnanomolar δ-receptor affinity. It also turned out to be highly stable against enzymatic degradation, unlike other δ-antagonists.

Salvadori *et al*[43] found that replacing the D-Ala-Phe amide bond in the dermorphin tetrapeptide Tyr-D-Ala-Phe-Gly-$NH_2$ with the reduced amide isostere gave a 5-fold increase in μ-opioid receptor selectivity.

In the last few years, there have been many reduced peptide analogues prepared to inhibit HIV-1 protease. These include work by Kent *et al,*[44] who in 1989 showed that the reduced amide analogue *N*-Ac-Thr-Ile-Nle-ψ[$CH_2NH$]-Nle-Gln-Arg-$NH_2$, (MVT-101), bound to HIV-1 protease in an extended conformer. It was shown to be a poor inhibitor. The reduced amide inhibitor $H_3N^+$-Ser-Gln-Asn-Phe-ψ[$CH_2NH$]-Pro-Val-Val-Gln[45] was also shown to have low potency. Sakurai, Nishigaki and Yake *et al*[46] prepared reduced peptides inhibitors of HIV-1 protease using a new methodology, (**Scheme 5**). They prepared four reduced amide analogues.




a. $MeSO_2Cl$, pyridine, 0°C, 97%; b. NaI, acetone, reflux, 69%; c. Pro-O$^t$Bu, $Na_2CO_3$, DMF, 80°C, 71%; d. 4M HCl, dioxane, RT, 85%; e. HCl.Gln-Ile-OMe or HCl.Ile-Val-OMe, DPPA, $Et_3N$, DMF, 0°C; f. i. Pd/C, $H_2$, MeOH; ii. Boc-Asn, DEPC, $Et_3N$, DMF, 4°C; g. i. 4M HCl, dioxane, RT; ii. Boc-Phe or Boc-Gln, DEPC, $Et_3N$, DMF, 4°C.

**Scheme 5**

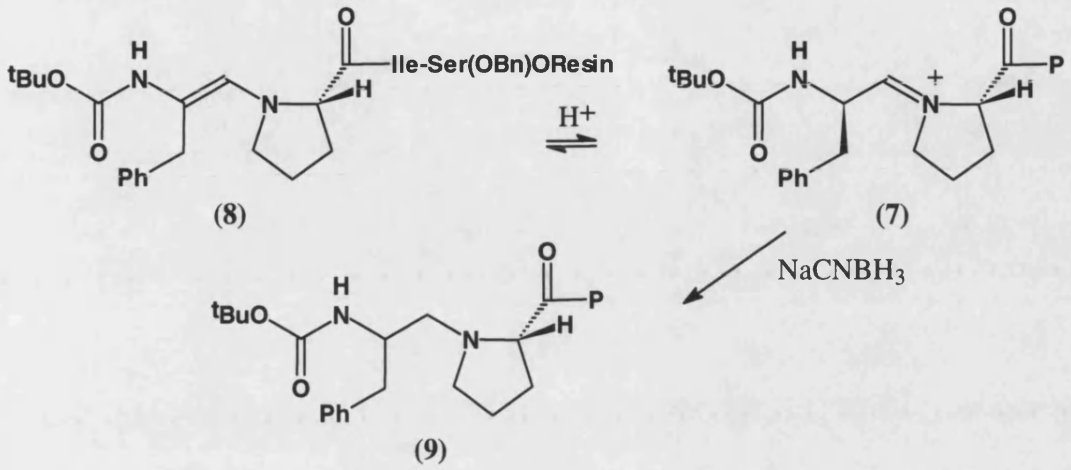
HIV-1 encodes its own protease which proteolytically processes precursor *gag* and *pol* proteins to form the native proteins needed for production of infectious viral particles. There are 8 possible cleavages sites, shown in bold, of which 3 were examined. There were : p17-p24 sequence Ser-Gln-Asn-**Tyr-Pro**-Ile-Val-Gln; p7-p6, Pro-Gly-Asn-**Phe-Pro**-Gln-Ile-Thr; and -PR (HIV-PR is the encoded protease responsible for processing *gag* and *gag-pol* gene products), Thr-Leu-Asn-**Phe-Pro**-Ile-Ser-Pro. They examined the effect of polarity on $p_3$ and $p_{2'}$ sites. They found that all four were weaker inhibitors than pepstatin A, an inhibitor of HIV-1 protease, probably due to the absence of the hydroxyl group, which is crucial for the interaction with the catalytic aspartic acids of the active site.

Reduced amide isostere analogues have also been prepared to examine binding to DNA,[47] and extensive conformational studies using n.m.r.[48] and molecular modelling techniques have been used to examine H-bonding, H-donor potentials of ψ [$CH_2NH$],[49] β-turns, α-helices, parallel and anti-parallel β-sheets.[50]

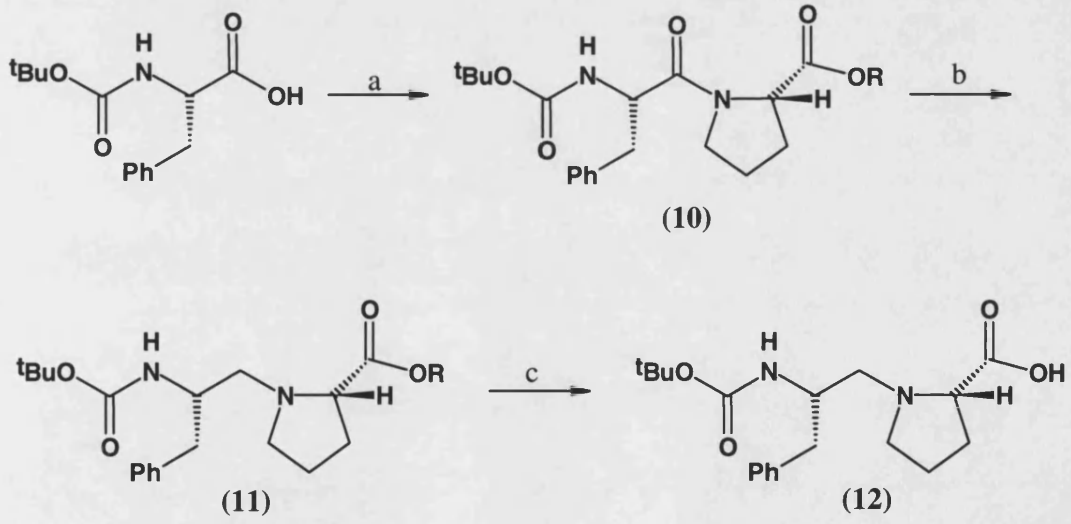
More recently, new techniques have been developed to prepare reduced amide isosteres. Cushman *et al*[51] found that reduction of *N*-methoxy-*N*-methylamides to the corresponding aldehyde, did not always give enantiomerically pure aldehydes. Even at -80°C using 0.8 eq. $LiAlH_4$, the aldehyde was produced with only 88% enantiomer excess (e.e.). They could not achieve higher optical purities and the racemisation was connected to the reductive alkylation step. As they used proline, a secondary amine, they postulated that the imine **(7)** was in equilibrium with enamine **(8)**, **Scheme 6**.

They developed a new synthetic route. This involved the formation of the dipeptide **(10)** which was then reduced by diborane, to the reduced amide analogue **(11)**, **Figure 5**. The reduce amide dipeptide analogue **(12)** was then coupled using DCC, HOBt, DMF, with resin bound Ile and the resultant pseudotripeptide was cleaved from the

resin using standard solid phase methodology. Veki, Myanoto and Oyanada[52] have reported improvements in this method.



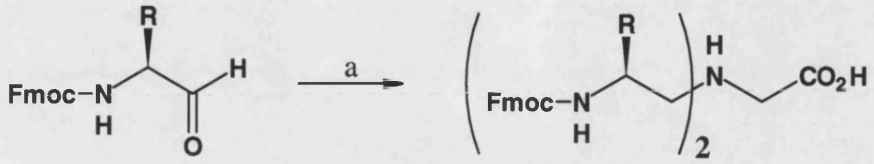

**Scheme 6**




a. Pro-OR, BOP, $Et_3N$, $CH_3CN$; b. $B_2H_6$, THF; c. i. 1M NaOH, MeOH; or ii. $H_2$, Pd/C, MeOH.

**Figure 5**

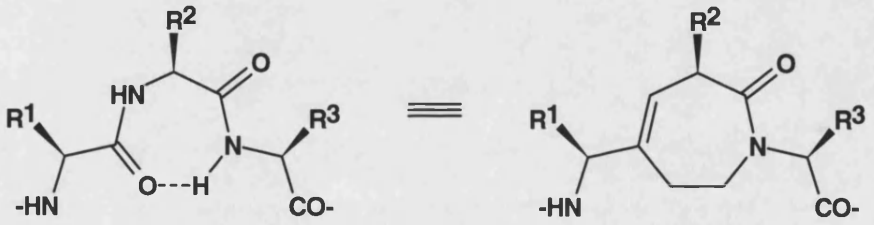
Salvi *et al*[53] also encountered problems using the reductive amination method for preparing reduced amide isosteres. They discovered double condensation products, see **Figure 6** were generated when they performed reductive amination with cyanoborohydride.




R = H or *p*-$(CH_3)_3COC_6H_4CH_2$
a. $NaCNBH_3$, MeOH; AcOH (99:1), RT, Gly.

**Figure 6**

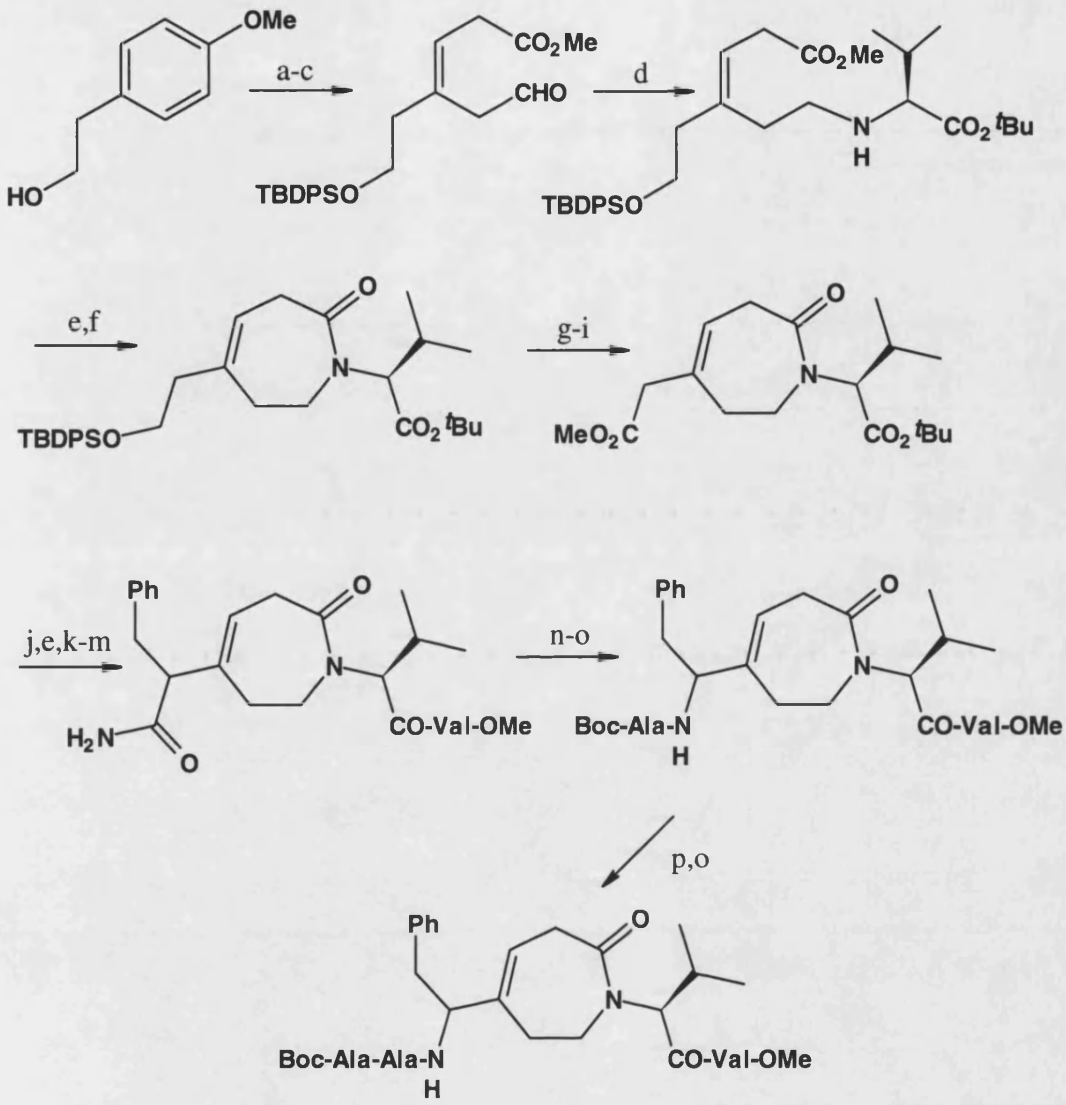
Newlander *et al*[54] developed an unusual constrained reduced amide inhibitor of HIV-1 protease from the sequential incorporation of γ-turn mimetic into the substrate, **Figure 7**.




γ-turn mimetic constrained reduced amide

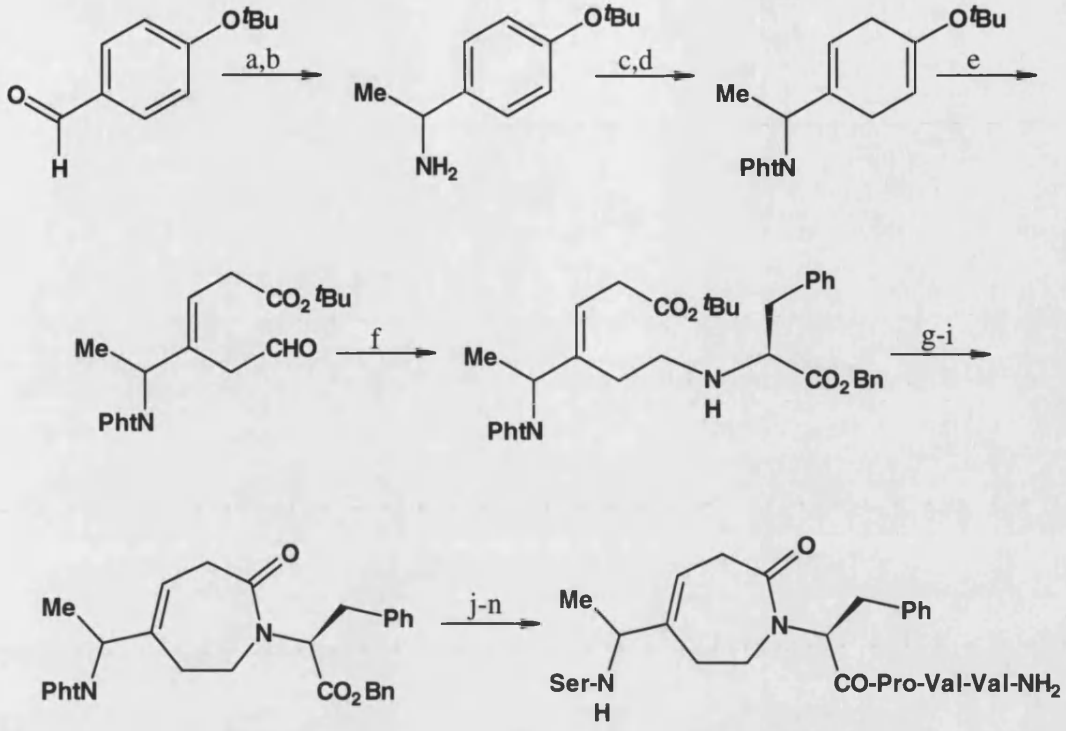
**Figure 7**

They prepared the inhibitors from 4-methoxyphenethyl alcohol **(13)** following the experimental procedure in **Scheme 7**, and an alternative route in **Scheme 8**.




a. Li, $NH_3$, $^tBuOH$, EtOH; b. TBDPSCl, imidazole, DMF; c. $O_3$, MeOH, DMS; d. HCl-Val-O$^t$Bu, $Et_3N$, $NaCNBH_3$, MeOH; e. aq. NaOH, dioxane, HCl; f. DPPA, $Et_3N$, DMF; g. TBAF, THF; h. Jones oxidation; i. $CH_2N_2$, $Et_2O$; j. $KN(TMS)_2$, THF, BnBr; k. EtOCOCl, $Et_3N$, THF, $NH_4OH$; l. TFA, DCM; m. Val-OMe, DCC, HOBt, DMF, DCM; n. $PhI(OCOCF_3)_2$, $H_2O$, $CH_3CN$; o. $(Boc\text{-}Ala)_2O$, $Et_3N$, DMF; p. HCl, dioxane.

**Scheme 7**




a. $LiN(TMS)_2$, THF, $0^oC$; b. MeMgBr, reflux; c. Li, $NH_3$, THF, $^tBuOH$, EtOH; d. *N*-(ethoxycarbonyl)phthalimide, $Et_3N$, THF; e. $O_3$, DCM, MeOH, DMS; f. TSA-Phe-OBn, $Et_3N$, $NaCNBH_3$, MeOH; g. TFA, DCM; h. HCl, dioxane; i. DPPA, $Et_3N$, $NaHCO_3$, DMF; j. HF, $0^oC$, 45 mins.; k. TFA-Pro-Val-Val-$NH_2$, $Et_3N$, HOBt, BOP, DMF, $O^oC$, 2 days; l. $NH_2NH_2$-$H_2O$, EtOH; m. Boc-Ser, HOBt, DCC, DMF; n. TFA, DCM.

**Scheme 8**

Their model of the conformationally constrained reduced amide showed an 300-fold improvement in the binding affinity to HIV-1 protease, over the native amide.

Shuman *et al*[55] developed an inhibitor of thrombin which contained a reduced amide isostere. They found this analogue exhibited little or no ability at inhibiting serine proteases.

Another unusual approach to preparing reduced amide analogues **(13)** was reported by Nicolaides *et al.*[56] They prepared the analogues using standard reductive alkylation methodology and from aziridines **(14)**, **Figure 8**.


(14) (13)

a. amino ester, R=R"=alkyl.

**Figure 8**

### 1.4.2 Hydroxyethylene isosteres

#### 1.4.2.1 Renin Inhibitors

A major regulatory mechanism of the maintenance of blood pressure in mammals is the renin-angiotensin system (RAS). This involves a sequential enzymatic transformation of angiotensinogen to angiotensin I by renin, angiotensin I to angiotensin II by angiotensin converting enzyme (ACE) and angiotensin II to angiotensin III by aminopeptidase, **Figure 9**. The major pharmacological effects of AII are vasoconstriction and stimulation of the adrenal cortex to release aldosterone (AIII shows this to a lesser extent), this in turn induces sodium retention which results in hypertension. Possible modes of intervention in this cascade include inhibition of renin release, renin, ACE and aminopeptidase inhibition; and angiotensin II receptor antagonism.

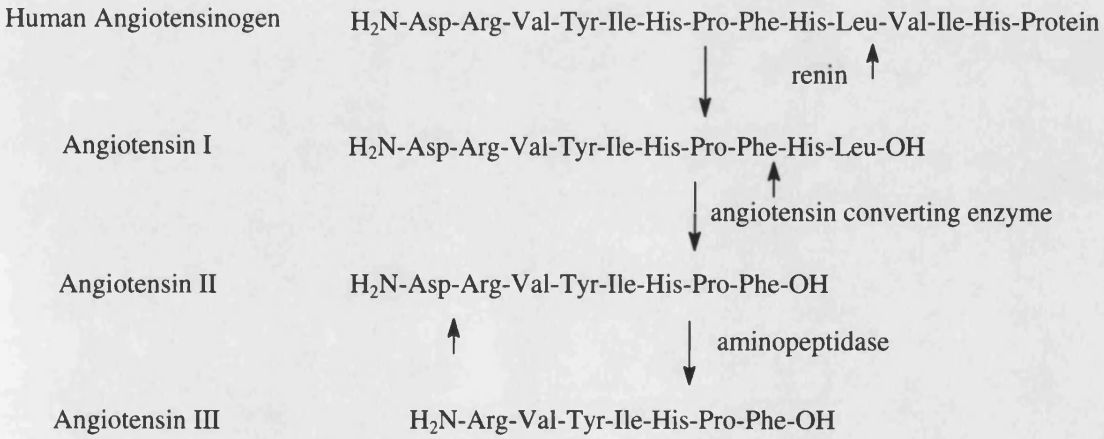
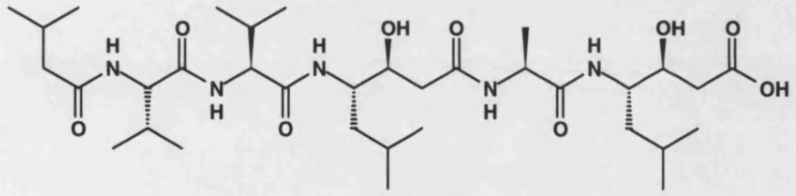


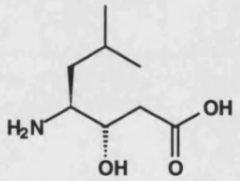

**Figure 9**

ACE inhibition has been an effective treatment for hypertension but unfortunately ACE is not specific for the substrate angiotensin I, since it also cleaves kinins and other endogenous peptides. This lack of specificity may contribute to the antihypertensive effects of ACE inhibitors. Inhibition of renin is by far a better method for the treatment of hypertension, due to fact that angiotensinogen is the only known naturally occurring substrate for renin.

The naturally occurring aspartic proteinase inhibitor pepstatin A, Iva-Val-Val-Sta-Ala-Sta; {Iva = $Me_2CHCH_2CO$ and Sta = (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid}, is a potent inhibitor of pepsin[57] and a weaker inhibitor of human renin.[58] The central statine unit has been proposed to serve as a mimic of the transition-state for hydrolysis. With the hydroxyl group of statine in the 3*S* configuration, statine-derived inhibitors are 1000-fold more active than the corresponding 3*R* isomers.[59]



Pepstatin A



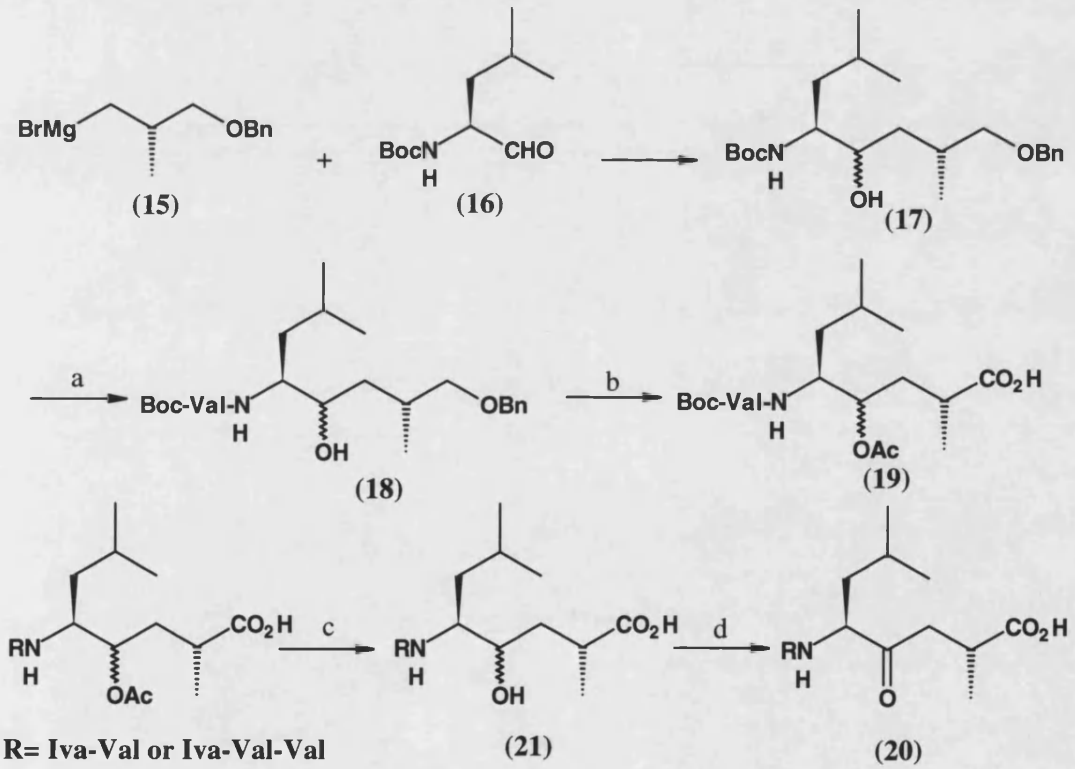
Statine

A second transition-state analogue introduced by the Szelke group, incorporates an hydroxy isostere ψ[$CHOH(CH_2)$] for the Leu-Val peptide bond. These analogues have proved to be potent inhibitors of renin.

As has been proposed for statine by Rich,[59] the hydroxy group of the isostere may serve as a mimic of the hydrated carbonyl of the tetrahedral intermediate in hydrolysis, while the Leu and Val side chains are correctly positioned to make binding interactions with the active site of renin. The development of this new type of transition-state analogue fuelled an immense amount of research into the inhibitory potency and activities of such analogues. These analogues have been used to inhibit

many proteinases such as aminopeptidase B, CCK-B, renin and HIV-1. This next section will be a review of all the literature on hydroxyethylenes since 1982.

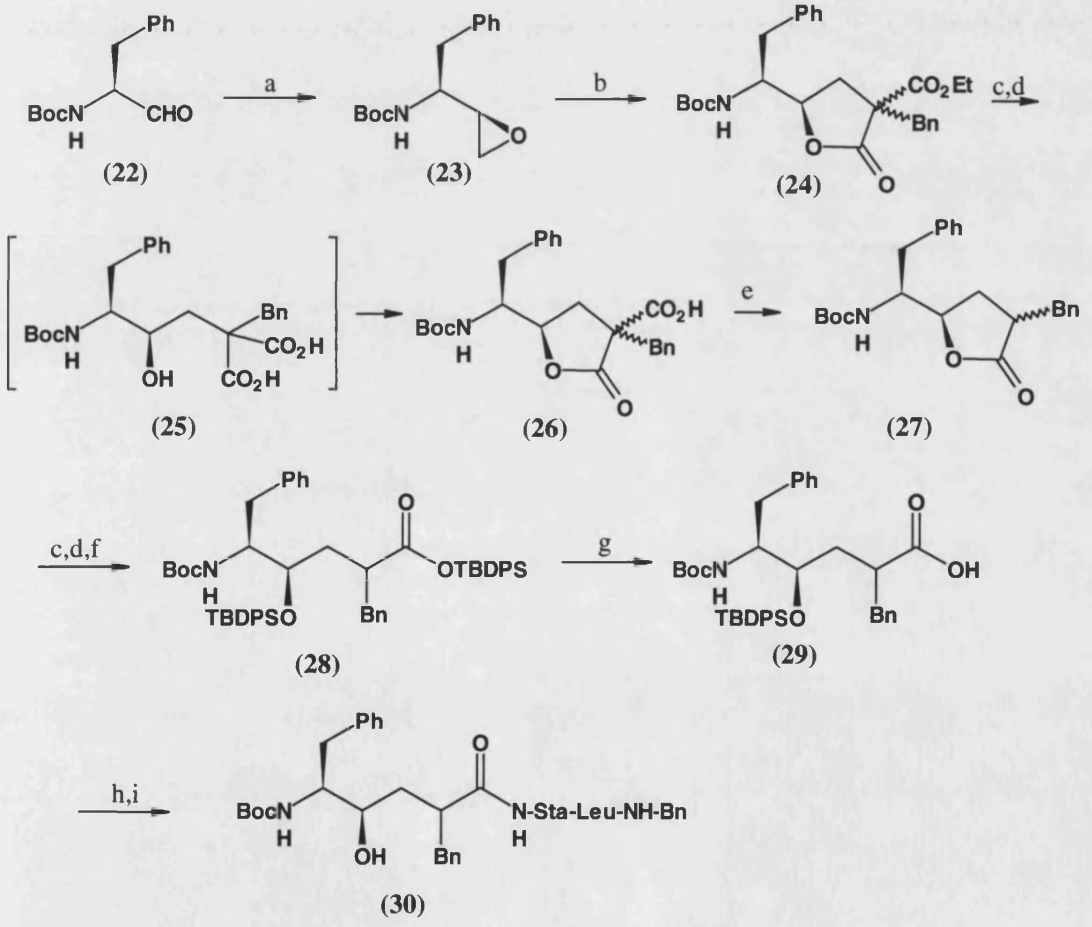
In a recent review, Greenlee[7] discussed the use of various transition-state analogues for the inhibition of human renin. As mentioned earlier Szelke[60] developed a second class of potent transition-state inhibitors in 1982.[11] Rich and Holloday[61] reported the preparation of hydroxyethylene and ketomethylene isosteres of Leu-Ala as shown in **Scheme 9**. This synthesis involved condensation of the Grignard reagent **(15)** with leucinal **(16)**, which furnished the alcohol **(17)** as a 4:1 diastereomeric mixture. The benzyl group **(18)** was converted to the carboxyl group **(19)** by debenzylation and pyridinium dichromate (PDC) oxidation. The ketomethylene **(20)** was prepared by oxidisation of the secondary alcohol **(21)** with PDC.




a. i. HCl, dioxane, ii. $(Boc\text{-}Val)_2O$; b. i. $Ac_2O$, DMAP; ii. $HCO_2NH_4$, Pd-C; iii. PDC, DMF; c. chromatography,. $K_2CO_3$, MeOH; d. PDC, DMF.

**Scheme 9**

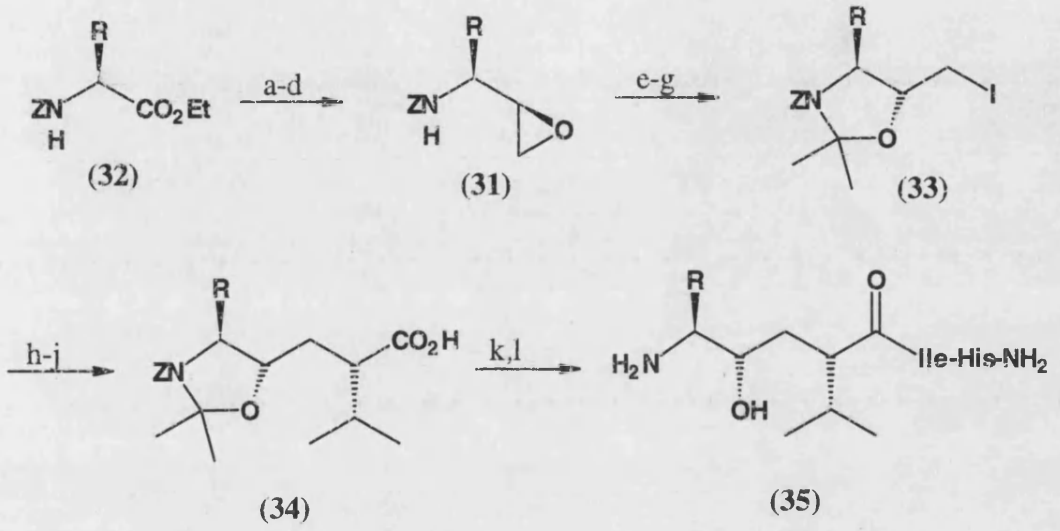
In 1985 Evans *et al*[62] prepared hydroxyethylene analogues from Boc-Phe-H **(22)**, **Scheme 10**. They reacted the aldehyde **(22)** with dimethylsulfonium methylide which gave the desired epoxide **(23)** in good yield. This was reacted with diethyl-2-benzylmalonate to give the lactone **(24)**. Aqueous base followed by aqueous acid, gave the open-chain acid **(25)** which cyclised spontaneously to give the carboxy lactones **(26)**. Decarboxylation gave lactone **(27)**. Aqueous base and aqueous acid wash of γ-lactone **(27)** followed by protection afforded the protected acid **(28)**. Removal of the silyl group from the carboxylate gave protected alcohol **(29)**. Coupling and deprotection furnished the hydroxyethylene analogue **(30)**.




a. $CH_2=SMe_2$; b. $C_6H_5CH_2CH(CO_2Et)_2$; c. NaOH; d. $H^+$; e. 120°C; f. TBDPSCl, imidazole; g. $H_2O$, AcOH, THF; h. Sta-Leu-NHBn, EDC, HOBt; i. TBAF, THF.

**Scheme 10**

In 1987, Stanton *et al*[63] prepared numerous hydroxyethylene isosteres in the hope of finding a potent inhibitor of renin. They prepared these analogues from homochiral *N*-protected amino epoxides (**31**) following the procedure detailed in **Scheme 11**.

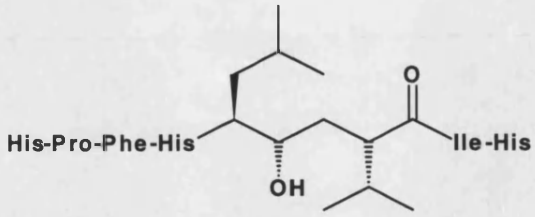



a. DiBAL-H; b. $H_2NNHCONH_2$; c. HCHO, HCl; d. $Me_2SOCH_2Na$; e. NaI, $Me_3SiCl$; f. KF; g. $(MeO_2CMe_2)TsOH$; h. $Me_2CHCOHLiCO_2Me$; i. $KO^tBu$, $H_2O$; j. chromatography; k. Ile-His-$NH_2$, DCC, HOBt; and l. deprotection.

**Scheme 11**

The epoxides (**31**) were prepared *via* reduction of the ester (**32**) to the aldehyde and then reaction with dimethyloxosulfonium methylide.[64] This produced the epoxides (**31**) as a 5:1 mixture of diastereoisomers, with the (2*R*,3*S*) isomer predominating. After ring-opening with iodotrimethylsilane, generated *in situ*, the diastereomeric alcohols were separated by chromatography. Acetonide formation led to protected aminoalcohol (**33**). Alkylation with methyl isovalerate, followed by hydrolysis gave the acid (**34**). The acid was then coupled to Ile-His-$NH_2$ using standard procedures, followed by deprotection to give the hydroxyethylene analogue (**35**). Stanton *et al*[63] incorporated this unit into a number of renin inhibitors, all showing $IC_{50}$ values between 2 and 20 nM.

Thaisrivongs *et al*[65] also prepared the hydroxyethylene isostere (**36**).

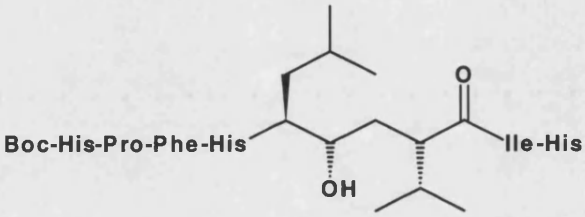



$IC_{50}$ (nM)

3

**(36)**

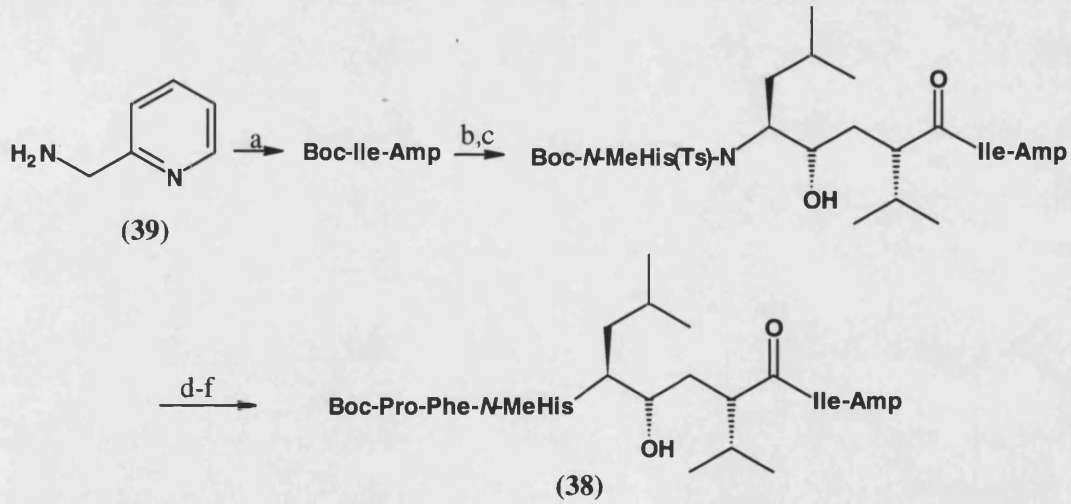
H-261




0.7

**(37)**

Day and Haber[66] reported that the *N*-Boc protected analogue H-261 (**37**) was more potent than the analogue prepared by Thaisrivongs *et al*.[65] Further modifications produced even more active inhibitors of renin, U-71,038[65] (**38**) with the *N*-methylated His and 2-(aminomethyl)pyridine (Amp) (**39**), **Scheme 12**.



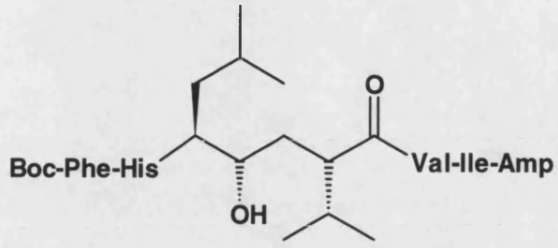

**(39)**

**(38)**

a. Boc-Ile, DCC, HOBt, 88%; b. TFA, DCM; Boc-Leu-ψ[CH(OTBDMS)$CH_2$]-Val, DEPC, $Et_3N$, 100%; c. $Et_2O$, HCl (g); Boc-*N*-Me-His(Ts)-OH, DEPC, $Et_3N$, 79%; d. TFA, DCM; Boc-Phe, DEPC, $Et_3N$, 98%; e. TFA, DCM; Boc-Pro, DEPC, $Et_3N$, 97%; f. HOBt, MeOH, 85%.

**Scheme 12**

Thaisrivongs *et al*[67] modified this renin inhibitor and produced the most active renin inhibitor in this series (**40**).



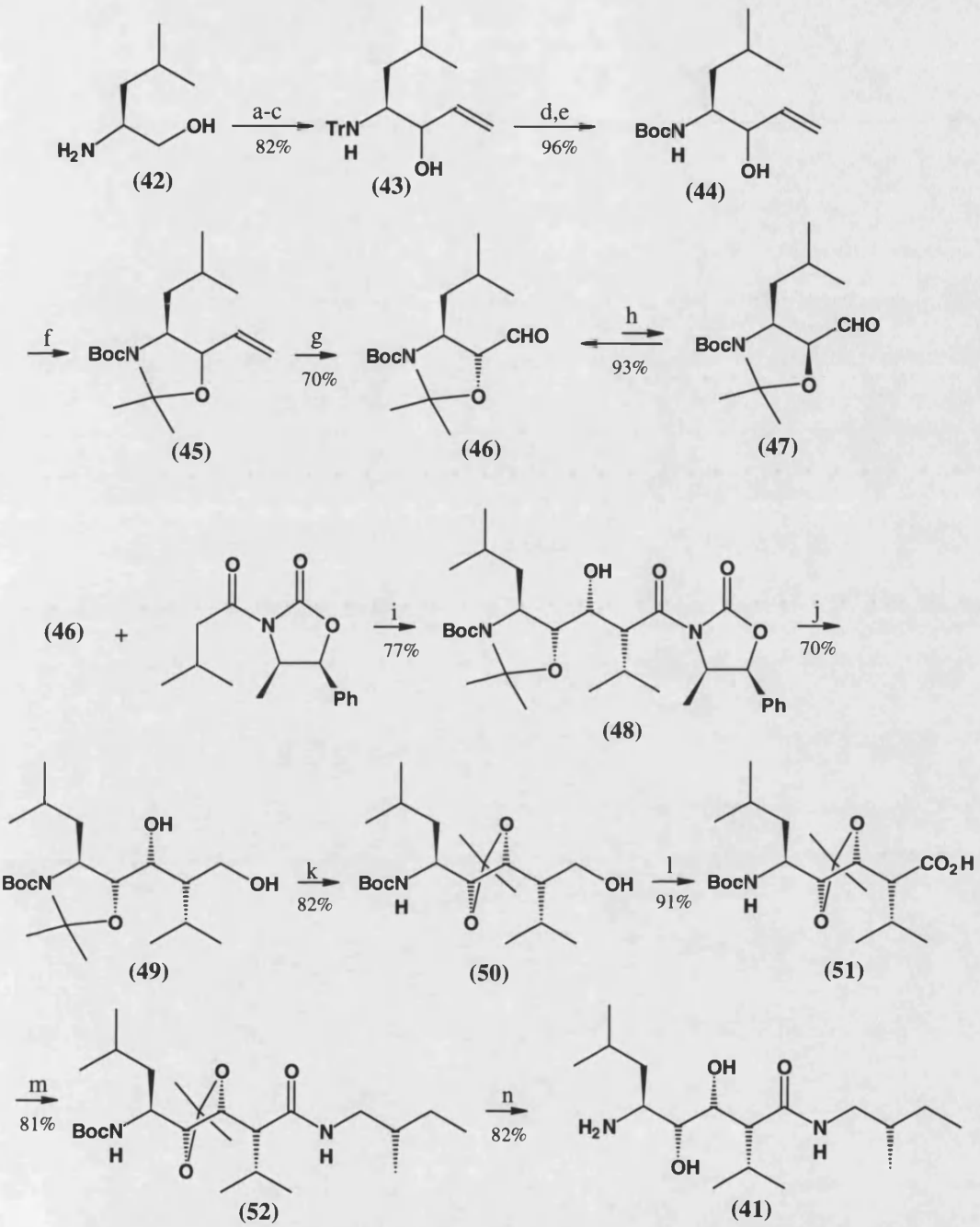

$IC_{50}$ 0.22nM

**(40)**

Thaisrivongs *et al*[67] also prepared a novel dihydroxyethylene isostere (**41**), **Scheme 13**.

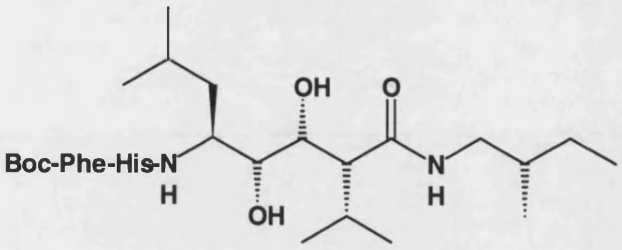
This preparation, started with leucinol (**42**) protection, oxidation and then Grignard addition, which gave the allylic alcohol (**43**) as a nearly equal mixture of diastereoisomers in good yield (82%). The protecting group was changed from the temporary trityl, to Boc (**44**). The trityl group was used in the vinylmagnesium bromide addition step, because the *N*-Boc protected route gave low yields with partial racemisation.

Protection of the amide and hydroxyl group gave (**45**), which was oxidised to the aldehyde (**46**). This was equilibrated under base conditions to give mainly aldehyde (**46**). Aldol addition of aldehyde (**46**) to acyl oxazolidinone (**47**) gave adduct (**48**) only as observed from $^{13}C$ n.m.r. spectroscopy. The chiral auxiliary was reductively removed to give the diol (**49**), which was protected via equilibration under acidic conditions to give alcohol (**50**). The primary alcohol was oxidised to acid (**51**) and coupled with 1-amino-2(*S*)-methylbutane to give the protected dihydroxyethylene isostere (**52**). This was deprotected under acidic conditions to afford the dihydroxyethylene pseudopeptide (**41**). This was incorporated into the inhibitors (**53**) and (**54**).




a. TrCl, $Et_3N$, DCM; b. $(COCl)_2$, DMSO, DCM; $Et_3N$; c. $CH_2$=CHMgBr, THF; d. DOWEX-50, W-X8, MeOH; e. $(Boc)_2O$, THF; f. $CH_2$=C(OMe)$CH_3$, *p*-TSA, DCM; g. $O_3$, DCM, MeOH; Zn, AcOH; h. $K_2CO_3$, MeOH; i. *n*-$Bu_2BOTf$, DIPEA, DCM, $H_2O$; j. *n*-$Bu_2BOTf$, DIPEA, THF, $LiBH_4$; k. Camphorsulfonic acid, DCM; acetone; l. $RuCl_3$, $H_2O$, $H_5IO_6$, $CH_3CN$, $CCl_4$; m. 1-amino-2-(*S*)-methylbutane, DEPC, $Et_3N$, DCM; and n. HCl, MeOH.

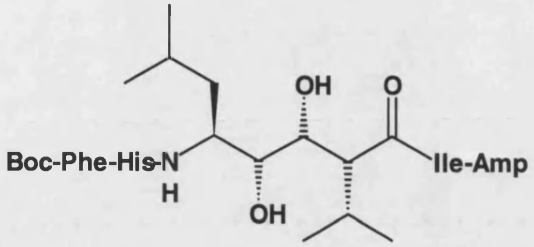
**Scheme 13**




(53)

$IC_{50}$ (nM)

3.4

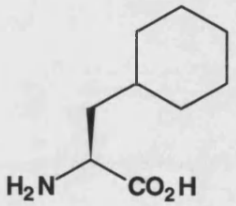



(54)

$IC_{50}$ (nM)

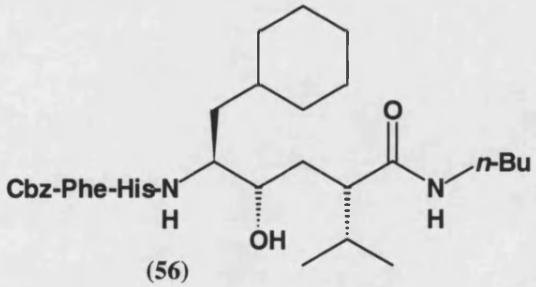
0.35

De Laszlo *et al*[68] modified the ($P^1$) site for human renin (leucine side chain) and produced the bulkier cyclohexylalanine (Cal) **(55)** derivative. This has been incorporated into a number of renin inhibitors.




**(55)**

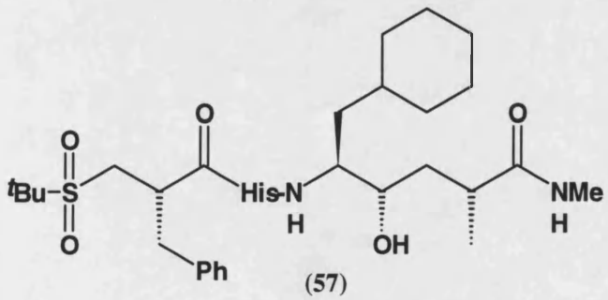
Stanton *et al*[69] explored the effect of modification of the $P^1$ site and found the most potent inhibitors to be **(56)** and **(57)**.




(56)

$IC_{50}$ (nM)

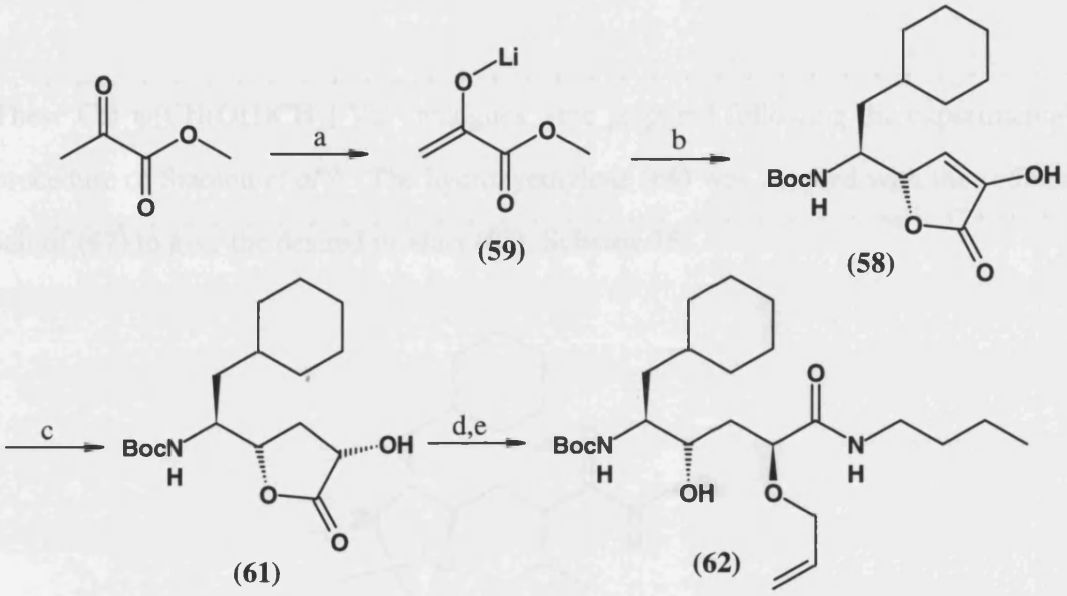
3




(57)

0.2

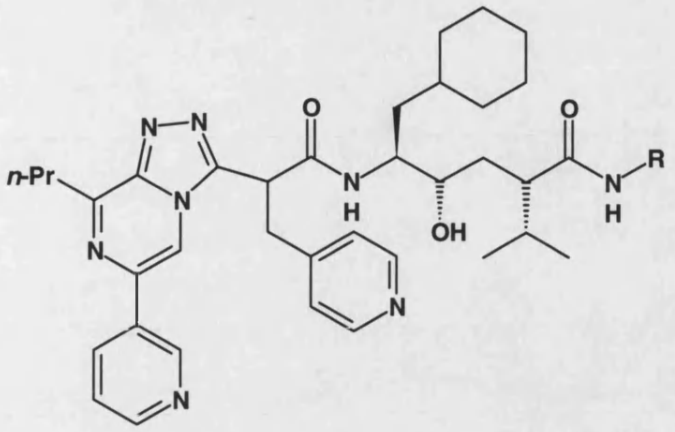
In 1988, Metternich and Lüdi,[70] prepared a novel hydroxy-γ-lactone **(58)** from the pyruvate enolate **(59)**. Chelation control of 1,2-induced asymmetric aldol addition of the pyruvate **(59)** to Boc-Cal-H **(60)** gave the corresponding hydroxy-δ-lactone **(58)** as the major diastereomer (80% d.e.). This was converted to the lactone **(61)** (59% overall yield), which was then ring-opened to the hydroxyethylene analogue **(62)** using allyl bromide and then butylamine, **Scheme 14**.

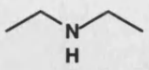



a. LDA, -78°C, THF:HMPA, (9:1), b. Boc-Cal-H (60), -78 → 0°C; c. $H_2$, Pd-C; HPLC; d. $CH_2$=$CHCH_2Br$, $Ag_2O$, $Et_2O$; and e. *n*-$BuNH_2$, RT.

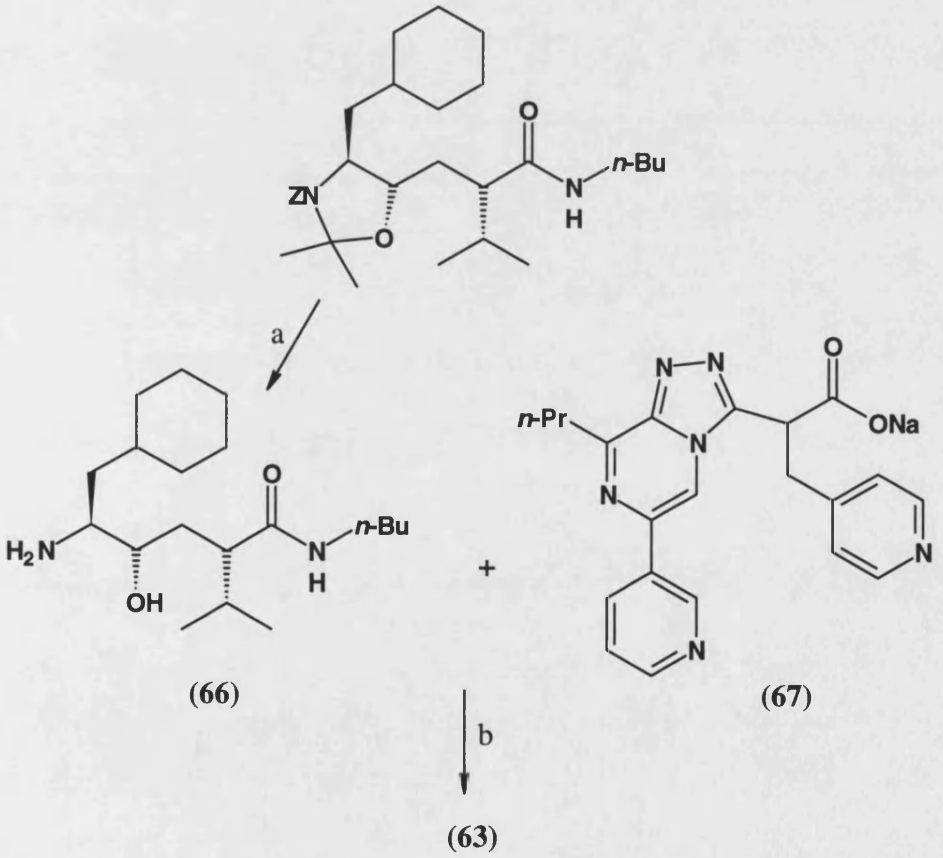
**Scheme 14**

Bradbury *et al*[71] expanded the use of the novel amino acid, Cal, and prepared several potent renin inhibitors. They incorporated non-peptidic portions for $P_4$-$P_2$ linked to the hydroxyethylene unit. The most potent were the *n*-Bu **(63)**, $CH_2CH_2NEt$ **(64)** and ω-hydroxypentyl **(65)** analogues.



| R | | $IC_{50}$ (nM) |
|---|---|---|
| **n-Bn** | **(63)** | **0.2** |
|  | **(64)** | **0.2** |
|  | **(65)** | **0.3** |

These Cal-ψ[CH(OH)$CH_2$]-Val analogues were prepared following the experimental procedure of Stanton *et al*[63]. The hydroxyethylene **(66)** was coupled with the sodium salt of **(67)** to give the desired product **(63)**, **Scheme 15**.



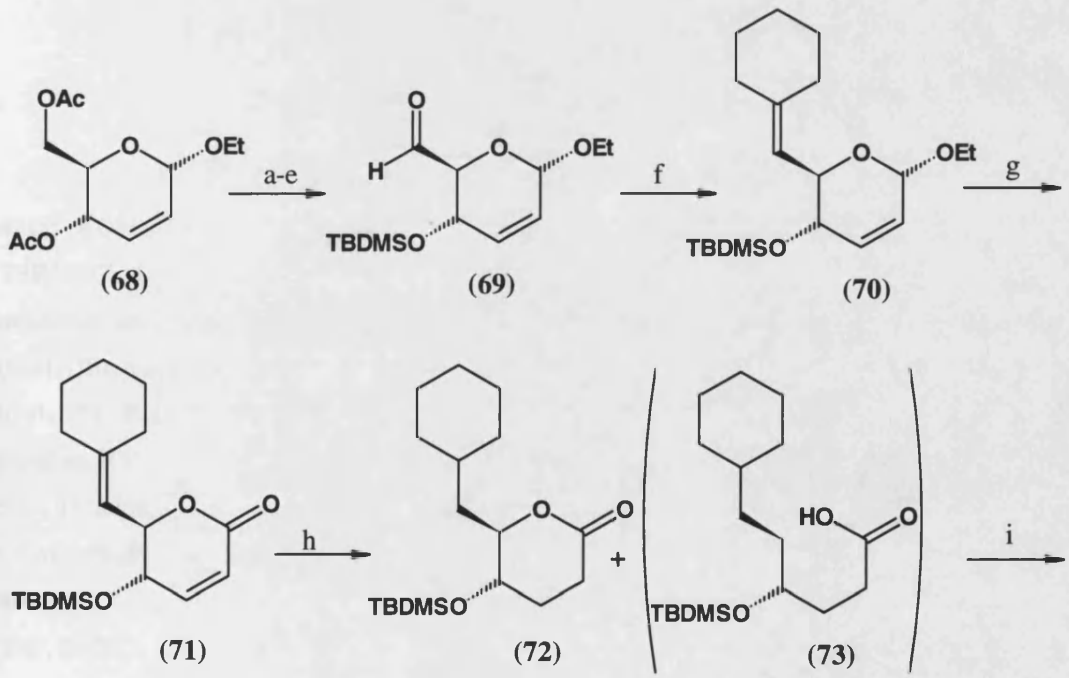
**(66)** **(67)** **(63)**

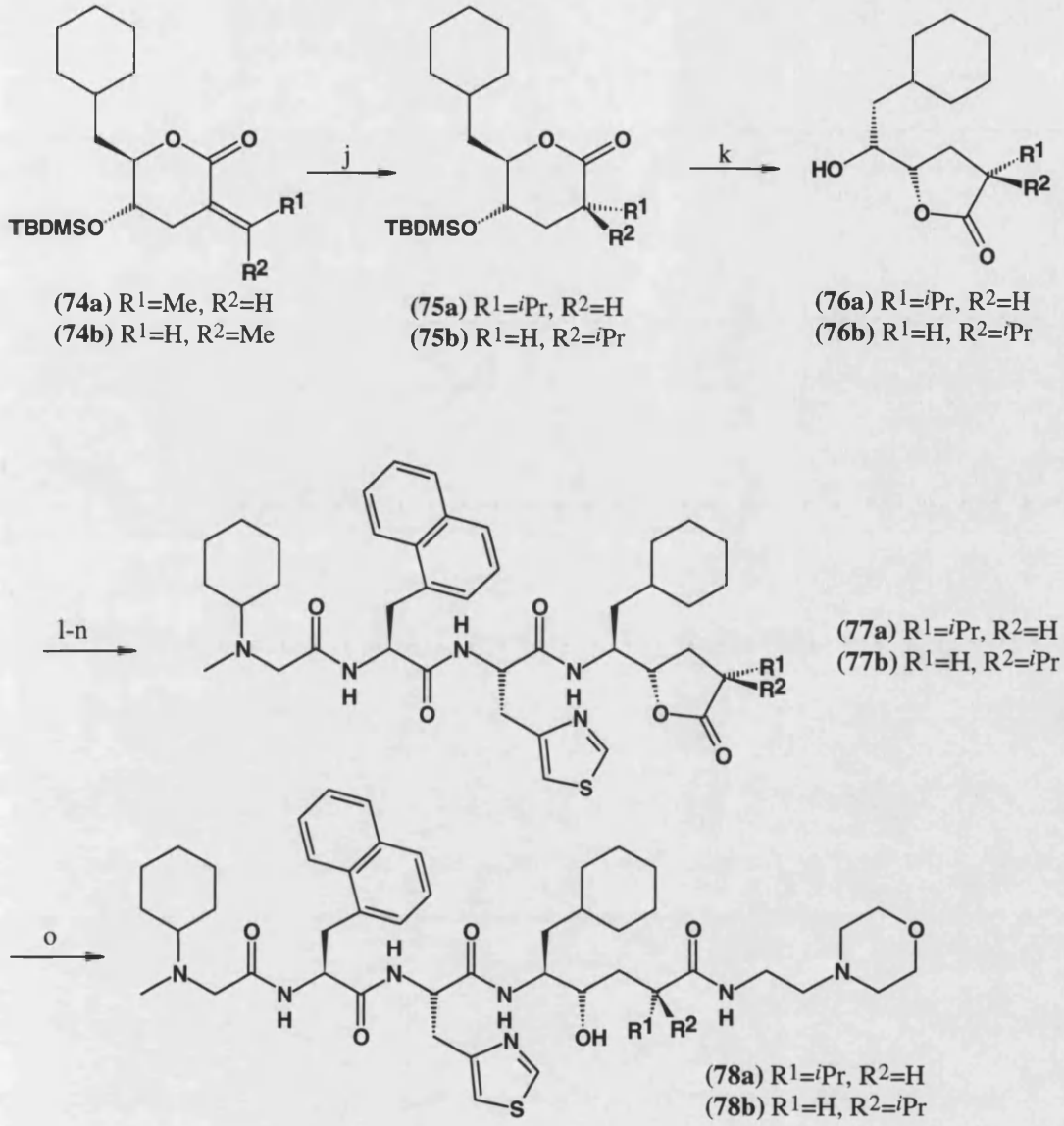
a. $NH_4O_2CH$, Pd/C, EtOH, $H_2O$; b. HOBt, HCl. $Me_2N(CH_2)_3N{=}C{=}NEt$, DMF

**Scheme 15**

Shiozaki and Kobayashi[72] prepared hydroxyethylene isosteres from 3,4,6-tri-*O*-acetyl-D-glucal. The use of the sugar reduced the number of diastereomers in the synthesis to two, whereas previous syntheses have produced at least four diastereomers.

3,4,6-Tri-*O*-acetyl-D-glucal was converted to the lactone **(68)**, this was transformed to the aldehyde **(69)**. Wittig reaction produced the diene **(70)**, and treatment of **(70)** in acetone with Jones reagent gave enone **(71)**. Hydrogenation of **(71)** gave the lactone **(72)** and the acid **(73)**. Lactone **(72)** was alkylated, to give a mixture of hydroxyethyl compounds. Mesylation and then elimination with 1,5-diazabicyclo [4,3,0] non-5-ene (DBN) gave an *E*:*Z* (88:5) mixture of *endo*-isomer (*Z*) **(74a)** and the *exo*-isomer (*E*) **(74b)**. Methylation of **(74)** with $Me_2CuLi$ gave a 1:1 mixture of diastereomers **(75a)** and **(75b)**. The unwanted isomer **(75a)** was converted to **(75b)** by equilibrating with $LiN(TMS)_2$, separating out the desired isomer **(75b)** and repeating the procedure with **(75a)**. Lactone **(75b)** was converted to γ-lactone **(76)**, which was transformed to tetrapeptide **(77)** and finally the lactone was reacted with 4-(2'-aminoethyl) morpholine to give the target compound **(78)**, **Scheme 16**.






**(74a)** $R^1$=Me, $R^2$=H
**(74b)** $R^1$=H, $R^2$=Me

**(75a)** $R^1$=*i*Pr, $R^2$=H
**(75b)** $R^1$=H, $R^2$=*i*Pr

**(76a)** $R^1$=*i*Pr, $R^2$=H
**(76b)** $R^1$=H, $R^2$=*i*Pr

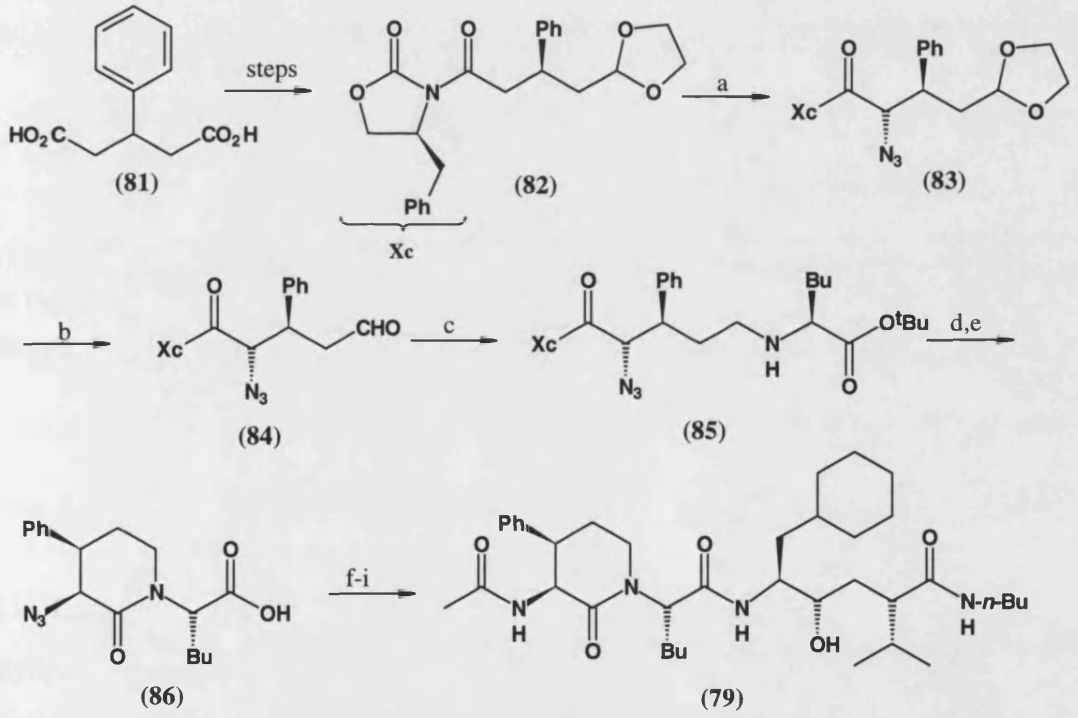
**(77a)** $R^1$=*i*Pr, $R^2$=H
**(77b)** $R^1$=H, $R^2$=*i*Pr

**(78a)** $R^1$=*i*Pr, $R^2$=H
**(78b)** $R^1$=H, $R^2$=*i*Pr

a. cat. KOH, EtOH, RT, 16 hrs.; b. pivaloyl chloride, pyridine-DMAP, RT, 16 hrs., THF, 85%; c. TBDMSCl, DMAP, DCM, reflux, 3 hrs., 98%; d. $LiAlH_4$, THF, 5$^{O}$C, 15 mins., 84%; e. PCC, 3Å molecular sieves, DCM, RT, 2 hrs., 56%, or DCC, DMSO, cat. $H_3PO_4$, RT, 16 hrs., 57%; f. $Ph_3P(C_6H_{11})Br$, $LiN(TMS)_2$, THF, reflux, 45 mins., 67%; g. Jones reagent, acetone, 0-5$^{O}$C, 10 mins., 57%; h. $H_2$, 5% Pd/C, EtOAc, RT, 86%; i. i. MeCHO, $LiN(TMS)_2$, THF, -78$^{O}$C, 15 mins.; ii $MeSO_2Cl$, pyridine, RT, 1 hr., iii. DBN, THF, RT, 10 mins., overall *E* = 78% and *Z* = 4%; j. $Me_2CuLi$, $Et_2O$, 0-5$^{O}$C, 15 mins., a = 49% and b = 49%; k. MeOH, HCl, reflux, 20 mins., a = 94% and b = 100%; l. DEAD, $Ph_3P$, DPPA, THF, 24$^{O}$C, 1 hr.; m. $H_2$, 10% Pd/C, EtOAc, RT, two steps, a = 95% and b = 87%; n. (*N*-cyclohexyl-*N*-methyl)glycyl-[3-(1-naphthyl)]-alanyl-[3-(4-thiazolyl)]-alanine, DEPC, $Et_3N$, THF, 24$^{O}$C, 1 hr., a = 56% and b = 60%; o. 4-(2-aminoethyl)morpholine, 80$^{O}$C, 10 hrs., a = 72% and b = 69%.

## Scheme 16

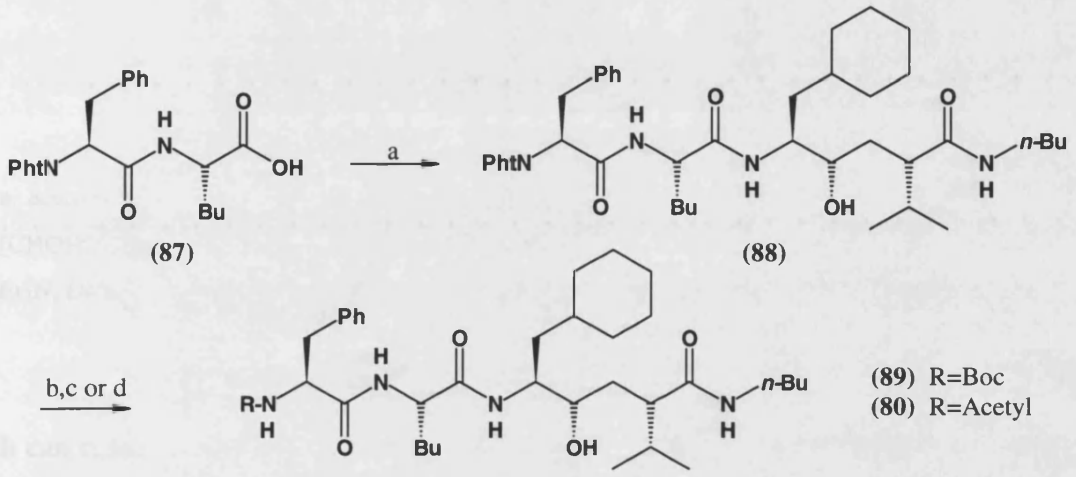
De Laszlo *et al*[73] used piperidine and benzazepinones in novel inhibitors of renin. They found the conformational constrained phenylalanine benzazepinone containing peptidomimetic **(79)** to be less active than the normal hydroxyethylene **(80)**. The hydroxylethylene pseudopeptide **(79)** was prepared from commercially available 3-phenylglutaric acid **(81)**. After several steps imide **(82)** was furnished. The imide **(82)** was converted to the azide **(83)** (90% e.e.). The acetal was hydrolysed to the aldehyde **(84)** which underwent reductive amination to give the amine **(85)**. Cyclisation at 110°C in DMF and hydrolysis of the *t*-butyl ester with HCl in EtOAc gave acid **(86)**. This was coupled, and the azide converted to the *N*-acetyl hydroxyethylene isostere **(79)**, **Scheme 17**.




a. $KN(TMS)_2$, THF, Tris-$N_3$, -78°C, 98%; b. AcOH, $H_2O$, THF, 90°C, 67%; c. $NaCNBH_3$, MeOH, Nle-O$^t$Bu, 3Å molecular sieves, 55%; d. DMF, 110°C, 84%; e. HCl, EtOAc, 100%; f. Cal-ψ [$CHOHCH_2$]-Val-NH-*n*-Bu, EDC, HOBt, DCM, 0°C, 50%; g. $HSCH_2CH_2SH$, $Et_3N$, MeOH, 57%; h. $Boc_2O$, DCM, 80%; i. $Ac_2O$, $Et_3N$, 70%.

**Scheme 17**

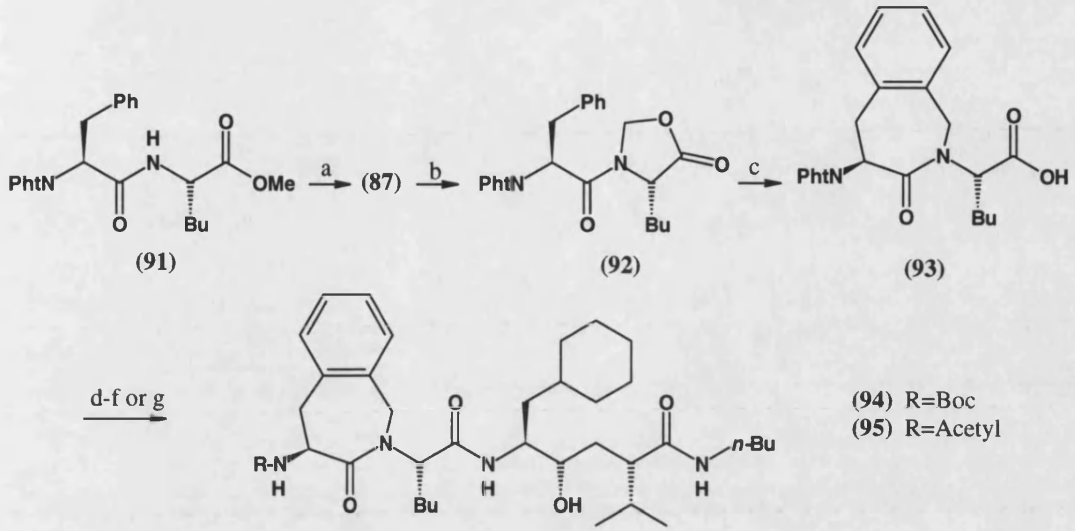
They also prepared the hydroxyethylene isostere **(80)**, **Scheme 18**, starting from *N*-phthaloyl protected dipeptide **(87)**, which coupled with Cal-ψ[$CHOHCH_2$]-Val-NH-*n*-Bu to give pseudotetrapeptide **(88)** in good yield (78%). Removal of the phthaloyl group and then protection gave the amino group with *N*-Boc **(89)** and *N*-acetyl **(80)**.




a. Cal-ψ[$CHOHCH_2$]-Val-NH-*n*-Bu, EDC, HOBt, DCM, 0°C, 78%; b. $N_2H_4$, EtOH, reflux, 94%; c. $Boc_2O$, $Et_3N$, DCM, 86%; or d. $Ac_2O$, $Et_3N$, DCM, 100%.

**Scheme 18**

De Laszlo *et al*[73] also prepared the conformationally restrained phenyl alanine hydroxyethylene **(90)**. The ester **(91)** was hydrolysed and the resultant acid **(87)** was condensed with formaldehyde to give the chiral oxazolone **(92)**. When the oxazolone **(92)** was treated with triflic acid the 3-aminobenzazephin-2-one **(93)** was formed in excellent yield (96%). The *N*-terminal amino group was deprotected and protected as before (**Scheme 18**), yielding both the *N*-Boc **(84)** and *N*-acetyl **(95)** derivatives, **Scheme 19**.




a. acetone, HCl, $H_2O$, 60%; b. $(CH_2O)_n$, TsOH, toluene, 63%; c. $CF_3SO_3H$, DCM, 96%; d. Cal-ψ [$CHOHCH_2$]-Val-NH-*n*-Bu, EDC, HOBt, DCM, $0^oC$, 78%; e. $N_2H_4$, EtOH, reflux, 94%; f. $Boc_2O$, $Et_3N$, DCM; or g. $Ac_2O$, $Et_3N$, DCM.

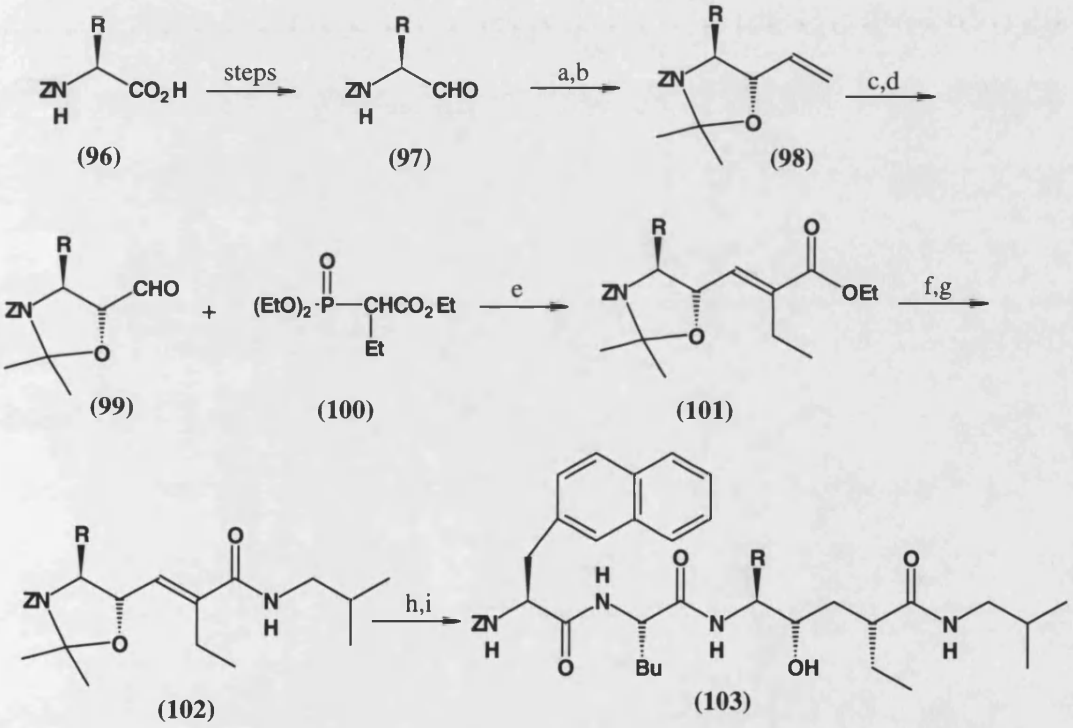
**Scheme 19**

It can clearly be seen in **Table 2** that in this series of peptidomimetics the smaller the *N*-protecting group the higher the potency, with *N*-acetyl showing the greatest activity.

**Table 2**

| Protecting group | compound | activity $IC_{50}$ (nM) |
|---|---|---|
| Acetyl | (79) | 21 |
| Acetyl | (80) | 0.84 |
| PhtN | (88) | 87 |
| Boc | (89) | 21 |
| PhtN | (93) | >20000 |
| Boc | (94) | 1730 |
| Acetyl | (95) | 210 |

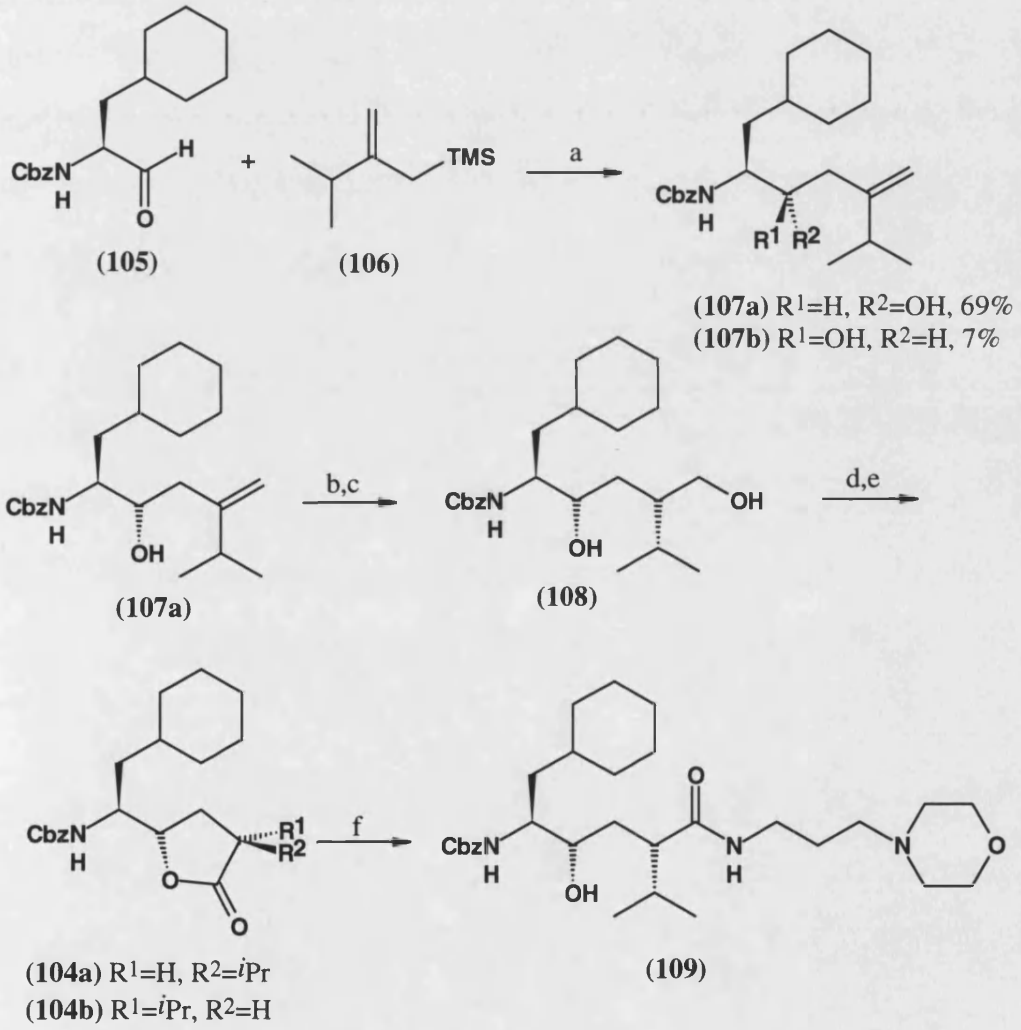
Atsuumi *et al*[74] prepared hydroxyethylene isosteres following a similar synthesis to that of Stanton *et al.*[63] They started from a variety of amino acids, which were converted to their corresponding Z-amino aldehydes (**97**). Reaction with vinyl magnesium bromide and then protection with 2,2-dimethoxypropane gave alkene (**98**). This was converted to the aldehyde (**99**) using $NaIO_4$-$OsO_4$ followed by equilibration with $K_2CO_3$ and MeOH to give mainly the *cis* aldehyde (**99**). Horner-Wittig-Emmons reaction of the aldehyde (**99**) with phosphonate (**100**) gave the alkene (**101**) in the ratio of 1:1 (*E:Z*). Conversion to the amide (**102**), followed by hydrogenation and coupling gave the hydroxyethylene analogue (**103**), **Scheme 20**.




a. $BrMgCH{=}CH_2$, b. 2,2-dimethoxypropane, *p*-TSA; c. $NaIO_4$, $OsO_4$; d. $K_2CO_3$, MeOH; e. LiCl, DBU; f. KOH; g. *iso*-$BuNH_2$, DPPA; h. $H_2$, (Pd-black); i. Z-Nal-Nle, DPPA. R = $CH_2Ph$ or cyclohexylethyl.

**Scheme 20**

Baker and Pratt[75] synthesised a hydroxyethylene analogue *via* a γ-lactone **(104)**, first introduced by Evans *et al.*[62] They prepared the γ-lactone **(104)** from the Cbz-Cal-H **(105)** by addition to (2-isopropylpropen-2-yl)trimethylsilane **(106)**. This gave the alkene **(107)**. Using borane-dimethylsulfide (BMS) the alkene was hydroborated, treatment with peroxide gave a mixture of diastereomers **(108)**. Oxidation with $RuCl_3$ and equilibration gave the γ-lactone **(104a)** as the major adduct. This was converted to the hydroxyethylene peptidomimetic **(109)** using 3-(4-morphiolin-4-yl)propylamine, **Scheme 21**.




**(105)** **(106)**

**(107a)** $R^1$=H, $R^2$=OH, 69%
**(107b)** $R^1$=OH, $R^2$=H, 7%

**(107a)** **(108)**

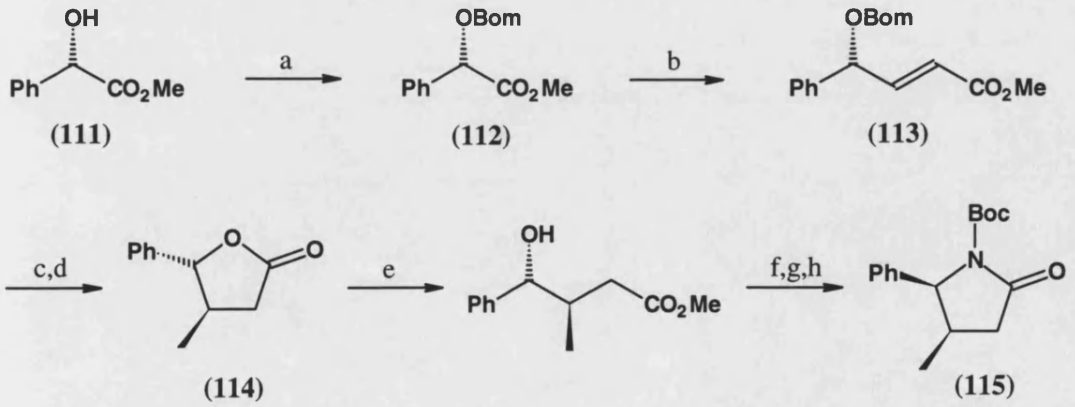
**(104a)** $R^1$=H, $R^2$=*i*Pr
**(104b)** $R^1$=*i*Pr, $R^2$=H

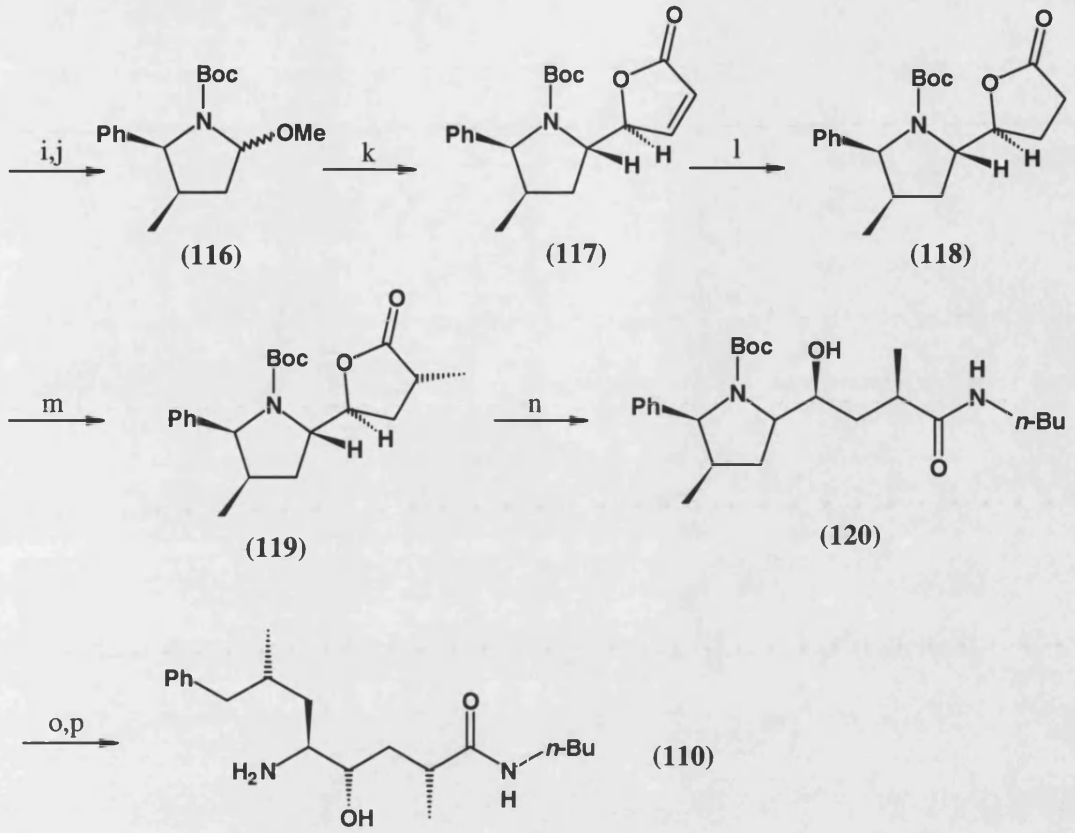
**(109)**

a. $SnCl_4$, DCM; b. BMS; c. NaOH, $H_2O_2$; d. $RuCl_3$, $NaIO_4$; e. MeOH, HCl (aq); f. 3-(4-morpholin-4-yl)propylamine, AcOH, 55°C.

**Scheme 21**

Hanessian and Raghaven[76] recently reported a novel synthesis of a hydroxyethylene analogue **(110)**. The synthesis started with chiral (*S*)-mandelic acid **(111)** which they used as a chiral template. The benzyloxymethyl (Bom) ether **(112)** was extended *via* a two-step process to give the α,β-unsaturated ester **(113)**. Organocuprate addition methylated the double bond with high stereocontrol, in which the *anti*-orientation predominated. Cleavage of Bom led to the lactone **(114)**. Hydrolysis, careful acidification, esterification and introduction of benzylic azido group using a Mitsunobu reaction, followed by reduction of the azido group with 1,3-propanedithiol, *N*-Boc protection then gave lactam **(115)**. Treatment of lactam **(115)** with diisobutylaluminium hydride (DiBAL-H) and trapping of the iminium ion with methanol under acid catalysis led to amino ether **(116)** as a mixture of anomers and rotamers. Lewis acid catalysed condensation of **(116)** with 2-(trimethylsilyloxy)furan gave the desired enone diastereomer **(117)** as the major adduct (6:1 *threo*:*erythro*). Hydrogenation of the double bond gave lactone **(118)**, which was methylated to give lactone **(119)**. Treatment of lactone **(119)** with dimethylaluminium butylamine gave the pyrrolidine **(120)**. Hydrogenolysis of **(120)** gave the *N*-Boc derivative of **(110)**, treatment with aq. HCl in dioxane gave the hydroxyethylene peptidomimetic **(110)**, **Scheme 22**.



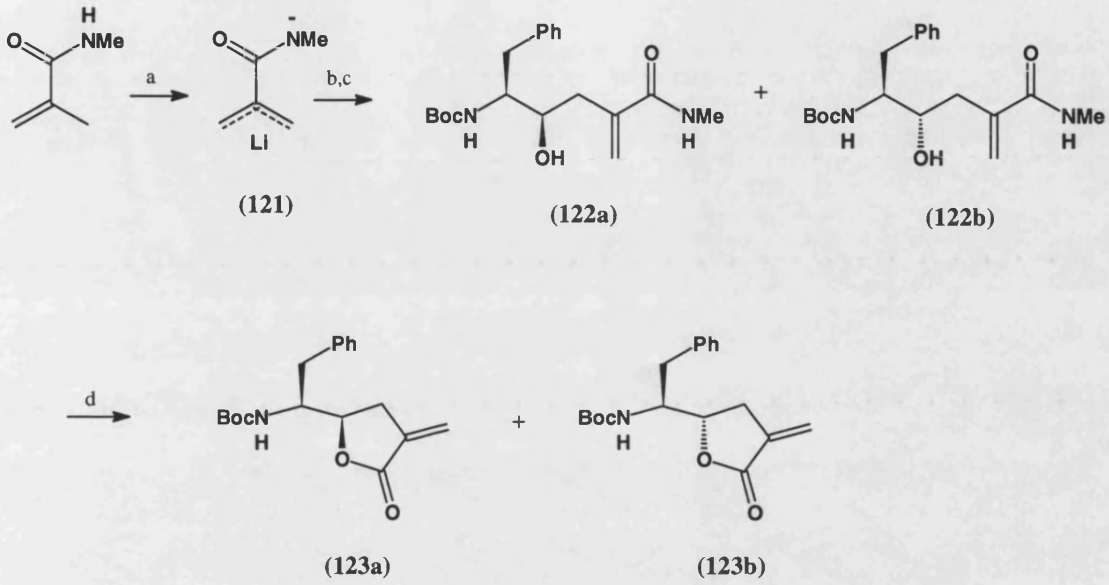



a) Bom-Cl, DIPEA, DCM, RT, 100 hrs., 83%, b) i. DiBAL-H, toluene, -78$^{o}$C, 3½ hrs.; ii. MeOH, -78$^{o}$C, 80 mins.; iii. methyltriphenylphosphoranylidene acetate, RT, 20 mins., 75% *trans*, 6% *cis*; c) $Me_2CuLi_2$.3TMSCl, THF, -78$^{o}$C, 2 hrs.; d) TMSBr, DCM, -23$^{o}$C to RT, 16 hrs., 72% for 2 steps; e) i. 0.5M NaOH, MeOH, 0$^{o}$C to RT, 2 hrs.; ii. 1M HCl; iii. $CH_2N_2$, $Et_2O$, EtOAc, 0$^{o}$C, 98%; f) DPPA, DEAD, $PPh_3$, THF, 0$^{o}$C to RT, 16 hrs., 89%; g) 1,3-propanedithiol, DIPEA, MeOH, RT, 48 hrs., 86%; h) $(Boc)_2O$, DIPEA, DMAP, DCM, RT, 24 hrs., 99%; i) i. DiBAL-H, toluene, -78$^{o}$C, 4 hrs.; ii. MeOH, -78$^{o}$C, 30 mins., 72%; j) CSA, MeOH, RT, 1 hr., 100%; k) 2-(trimethylsiloxy)furan, $BF_3.OEt_2$, DCM, -78$^{o}$C, 1 hr., (6:1, *threo*:*erythro*), 98%; l) $H_2$, 10% Pd/C, EtOAc, RT, 1 hr., 93%; m) i. $LiN(TMS)_2$, THF, -78$^{o}$C, 40 mins.; ii. MeI, -78$^{o}$C, 90 mins., -50$^{o}$C, 1 hr.; iii. AcOH, THF, 67%; n) $BuNHAlMe_2$, DCM, RT, 7 hrs., 76%; o) 20% $Pd(OH)_2$, $H_2$ (56 psi), EtOH:EtOAc (2:3), RT, 48 hrs., 73%; p) aq. HCl, dioxane, 0$^{o}$C, 2 hrs., 100%.

**Scheme 22**

Amino acids have been used as the chiral templates for a vast number of hydroxyethylene isostere syntheses. Kempf *et al*[77] developed a new method for the preparation of these analogues. Their synthesis involved the condensation of homochiral phenylaninal **(22)** with *N*-allylmethanylamide dianions **(121)**, **Scheme 23**,

to give the alcohols **(122a)** and **(122b)**. Lactonisation gave γ-lactones **(123a)** and **(123b)** in the ratio 1.6:1. Treatment of these γ-lactones **(123a)** and **(123b)** with the suitable amine yielded the corresponding hydroxyethylene isosteres.




**(121)** **(122a)** **(122b)**

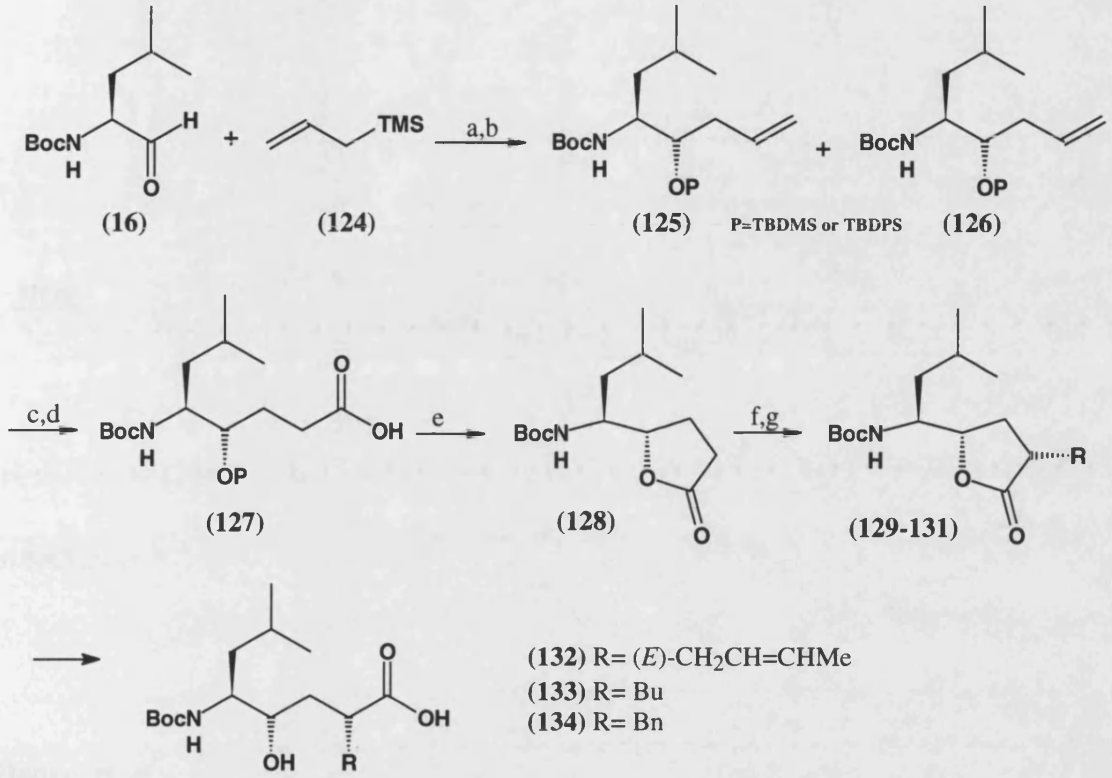
**(123a)** **(123b)**

a) *n*-BuLi, THF; b) $TiCl(O^iPr)_3$; c) Boc-Phe-H (22); d) xylene, reflux, (123a) : (123b), (1.6:1)

**Scheme 23**

Rich and Prasad[78] also used the diastereoselective allyl metal addition to α-amino aldehydes. They found that tin(IV)tetrachloride ($SnCl_4$) was the best Lewis acid for catalysis of the allyl trimethylsilane **(121)** condensation reactions. Condensation of Boc-Leu-H **(16)** with silane **(124)**, followed by protection, gave the alcohols **(125)** and **(126)** in the ratio 1:20.6, with the major isomer having the desired stereochemistry. Hydroboration followed by oxidation afforded the corresponding primary alcohol, which was oxidised to the acid **(127)**. Treatment with methanolic hydrogen chloride or tetrabutylammonium fluoride (TBAF) gave the lactone **(128)**. The lactone **(128)** was alkylated with crotyl, allyl or benzyl bromide to form the corresponding alkylated lactones **(129)**, **(130)** and **(131)** in excellent diastereomeric purities. The lactones

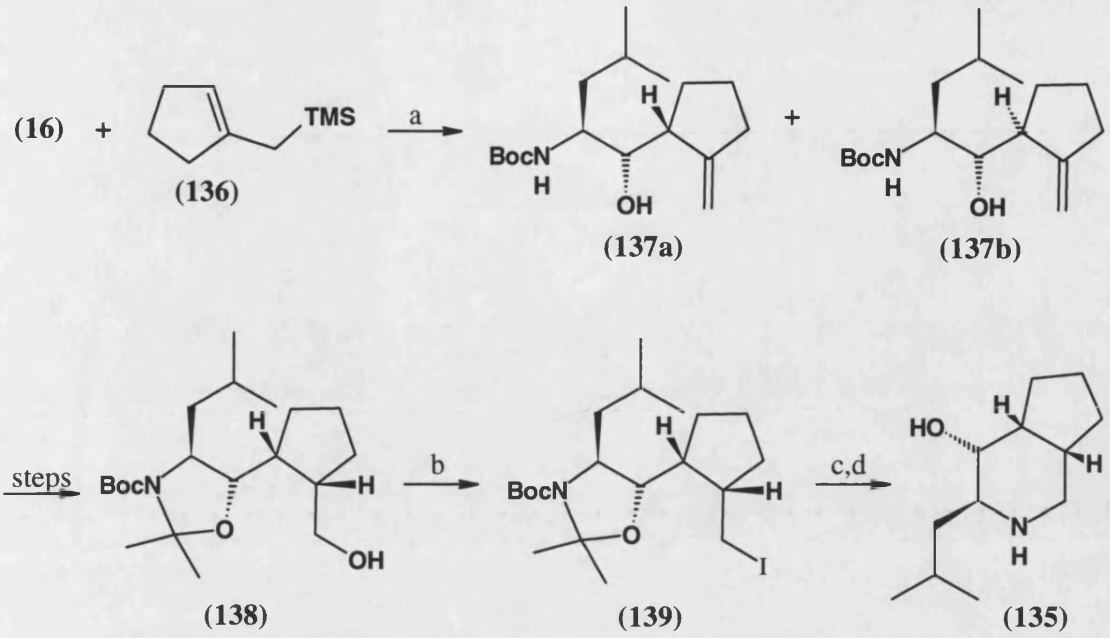
**(129)**, **(130)** and **(131)** were then converted to the corresponding hydroxyethylene analogues **(132)**, **(133)** and **(134)**, **Scheme 24**.




a) $SnCl_4$, -78°C, 4hrs; b) TBDMSCl or TBDPSCl, DMF, imidazole, RT; c) $BH_3$-THF, RT, 3hrs followed by NaOH, $H_2O$, RT, 6hrs; d) $RuCl_3$-hydrate, $NaIO_4$, $CH_3CN$:$CCl_4$:$H_2O$ (2:2:3), RT, 2hrs; e) 3M HCl in MeOH, RT, 1hr, or TBAF, THF, RT, overnight; f) $LiN(TMS)_2$, -78°C, 0.5hrs; g) *E*-crotyl bromide, BuBr or BnBr, -78°C, 2 hr.

**Scheme 24**

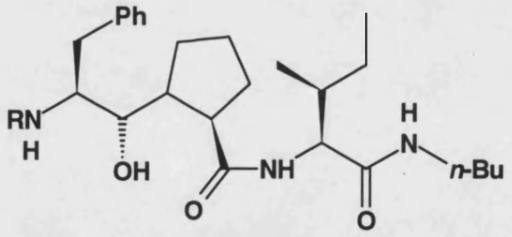
Rich and Prasad[78] used this methodology to prepare a restrained hydroxyethylene isostere **(135)**, which mimics a cyclic amino acid (*e.g.* proline). They prepared this analogue by reacting cyclopent-1-enyl-1-methylsilane **(136)** with Boc-Leu-H **(16)** as above. The alcohol **(138)** was converted to the iodide **(139)**, which underwent clean cyclisation to give the isostere **(135)**, **Scheme 25**.




a) $SnCl_4$, -78°C, 2hrs; b) $Ph_3P$, $I_2$, $CH_3CN$, $Et_2O$, 78%; c) 4M HCl in MeOH; d) $NaHCO_3$, DMF, 90%.

**Scheme 25**

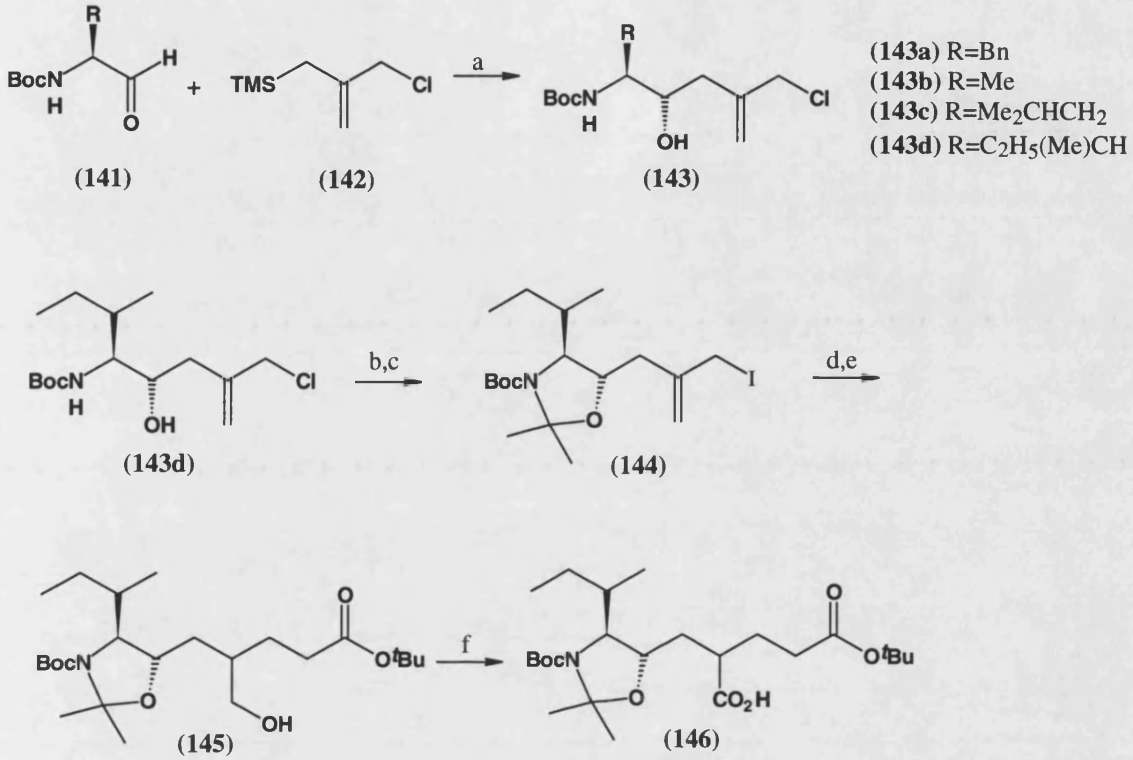
Hanko et al[79] prepared the hydroxyethylene isostere **(140)**, following a very similar method to Rich and Prasad.[78]




**(140)**

More recently, Taddei *et al*[80] and Kiyaooka *et al*[81] have used allyltrimethylsilanes to prepare hydroxyethylene isosteres. Taddei *et al*[80] found that *N*-Boc amino aldehydes **(141)** reacted with 2-chloromethyl-3-trimethylsilyl-1-propene **(142)** to give the alcohols **(143)** in good yields. Protection of the hydroxyl group **(143d)** and conversion of the chloride to the iodide **(144)** facilitated coupling with the lithium

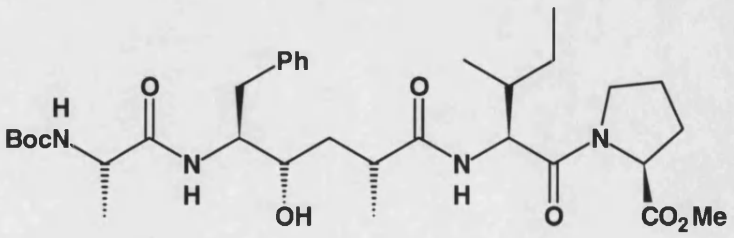
enolate of *tert*-butyl acetate. The alkene was converted to the alcohol **(145)** *via* hydroboration, followed by oxidation, which gave the acid **(146)**, **Scheme 26**.




a) $BF_3.OEt_2$, DCM, -60°C; b) $Me_2C(OMe)_2$, TsOH; c) NaI, acetone; d) LDA, $CH_3CO_2{}^tBu$; e) $BH_3$-THF, THF, 0°C followed by NaOH, $H_2O_2$; f) $PtO_2$, $O_2$.

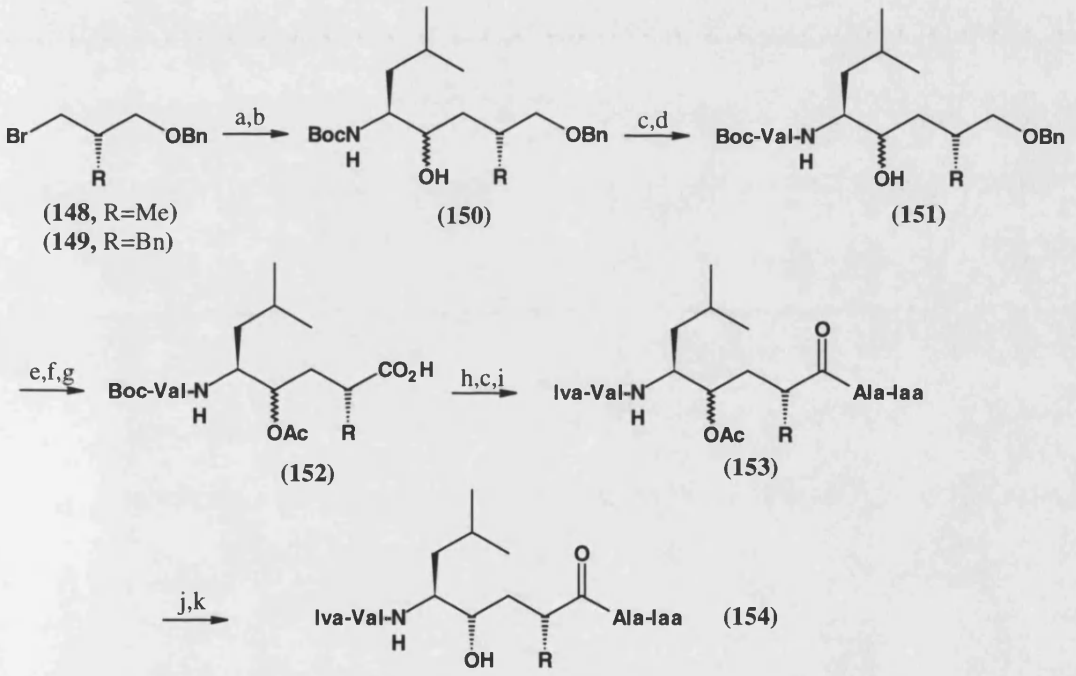
**Scheme 26**

Taddei *et al*[82] used $CrCl_2$ for the condensation step, and prepared the analogue **(147)**.




**(147)**

Rich *et al*[83] in 1987, prepared hydroxyethylene isostere utilising Grignard reagents, from the bromo ethers **(148)** and **(149)**. These reacted with Boc-Leu-H **(16)** to give the alcohols **(150)** in low yield (*ca.* 30% for R=Bn compared to 50-65% for R=Me). The *N*-terminus was extended by deprotection and coupling with Boc-Val to give **(151)**. Protection, then hydrogenation, followed by oxidation gave the acid **(152)**. Coupling with Ala-Iaa, (Iaa = amino acid) followed by deprotection of the *N*-terminus, and coupling with $(Iva)_2O$ gave the isostere **(153)**. After separation by chromatography and removal of the acetyl protecting group, the target hydroxyethylene isostere **(154)** was isolated, **Scheme 27**.

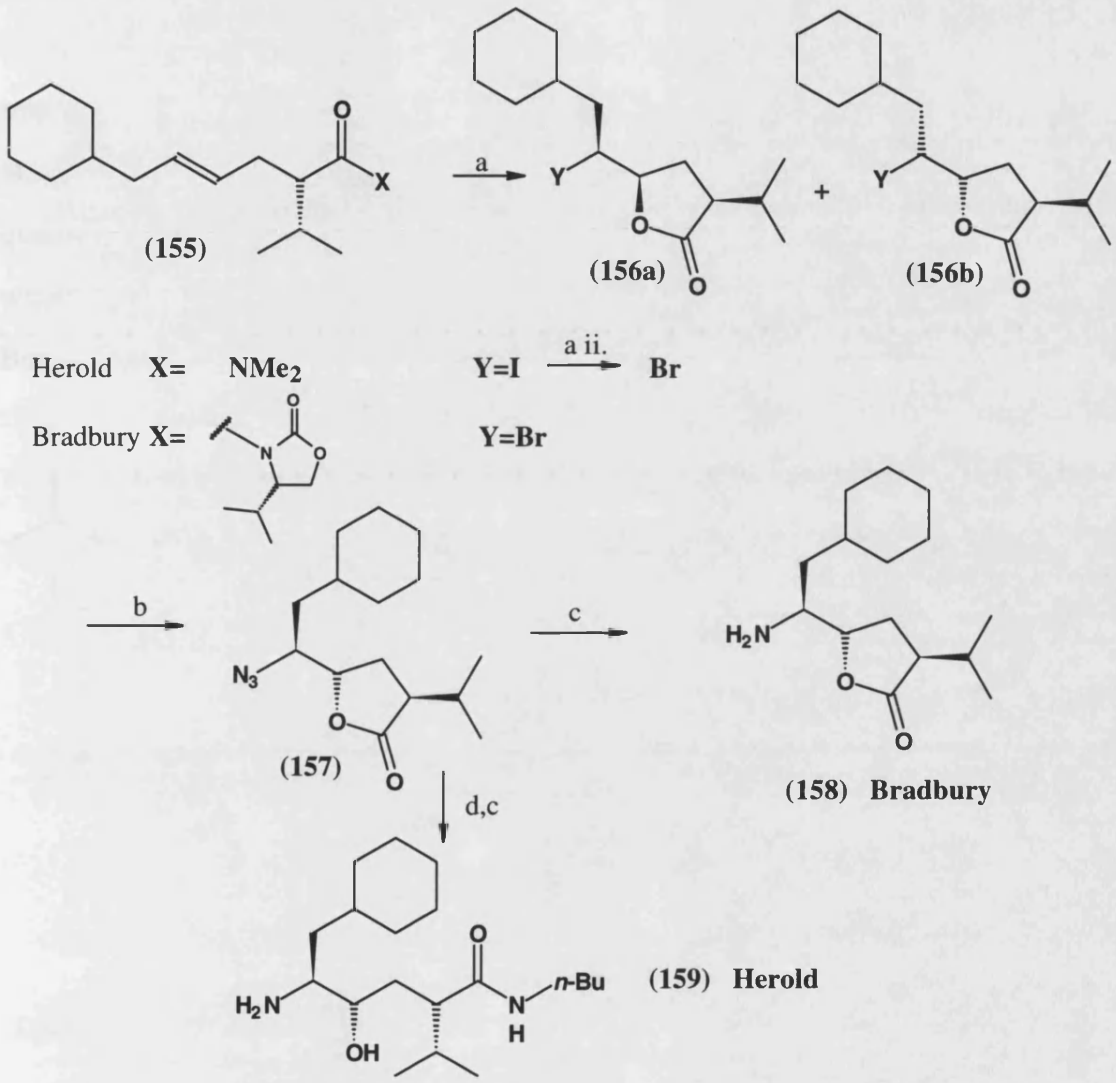



a) Mg, $Et_2O$; b) Boc-Leu-H (16); c) HCl, dioxane; d) $(Boc\text{-}Val)_2O$; e) $Ac_2O$, DMAP; f) $H_2$, Pd/C; g) $KMnO_4$, $C_6H_6$, $H_2O$, $Bu_4NBr$; h) Ala-Iaa, DCC, HOBt; i) $(Iva)_2O$; j) silica gel chromatography; k) MeOH, $K_2CO_3$.

**Scheme 27**

Herold *et al*[84] and Bradbury *et al*[85] both developed similar routes to hydroxyethylene isosteres utilising the Grignard reaction to prepare the intermediate alkene **(155)**. Halogenation of the double bond yielded the corresponding lactones **(156)**. The

bromide groups was converted to the azide **(157)**, which was then reduced to the amines **(158)** and **(159)**, **Scheme 28**.

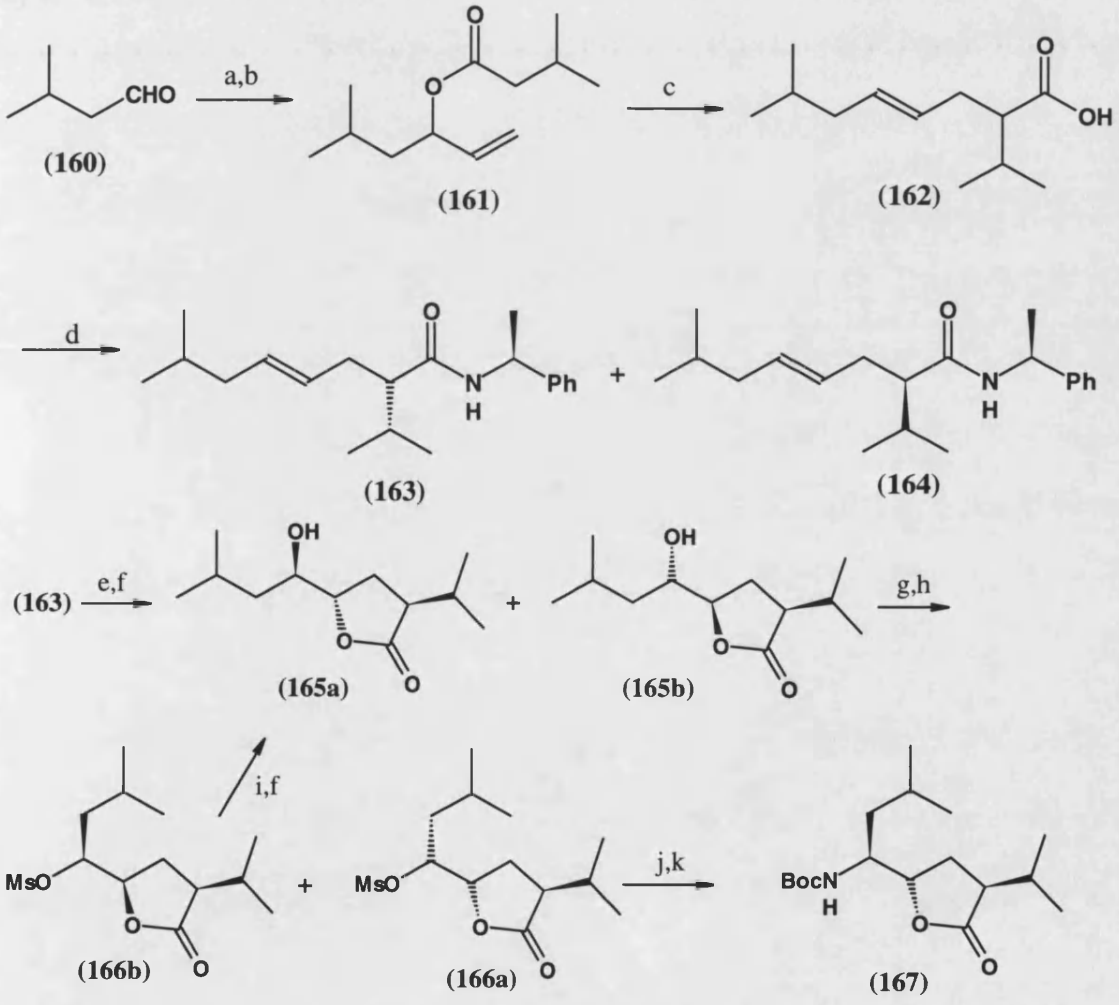



a) i. $I_2$, AcOH, THF, $H_2O$, 0°C or ii. NBS, $H_2O$, DME, 0-20°C; b) $NaN_3$, DMPU, 20°C; c) $H_2$, Pd/C, EtOH; d) *n*-butylamine, 40°C.

**Scheme 28**

Wuts *et al*[86] also utilised the Grignard reaction to assemble the backbone of the hydroxyethylene isostere of Leu-Val. Starting from 3-methylbutyraldehyde **(160)**, addition of vinylmagnesium bromide and subsequent acylation gave the ester **(161)**. Ireland-Claisen enolate rearrangement gave the acid **(162)**. The acid was converted to

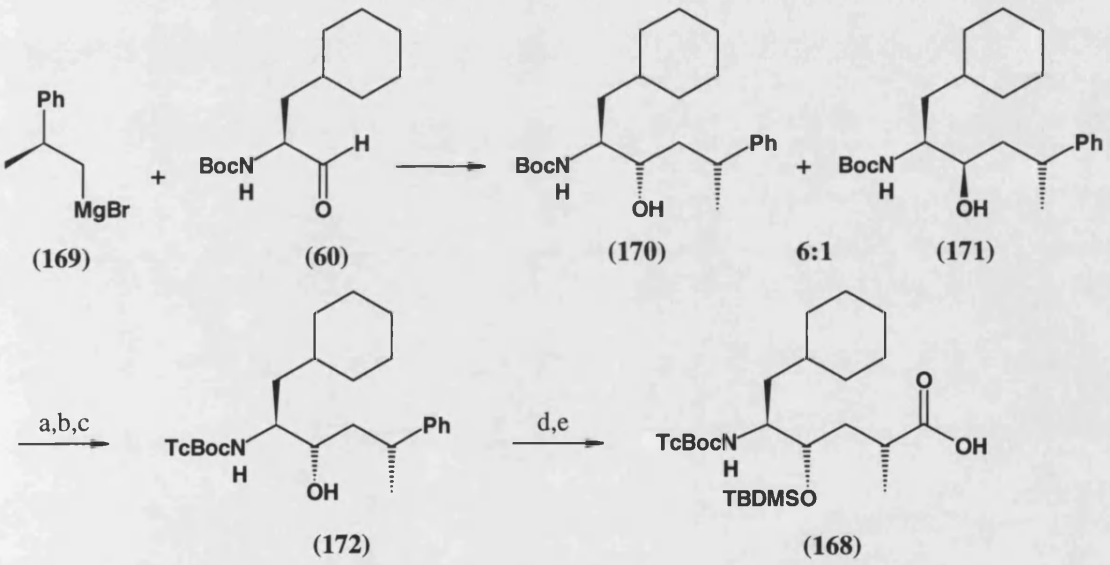
the diastereomeric amides **(163)** and **(164)**. Selective crystallisation afforded amide **(163)** in 98% d.e. Epoxidation of the alkene followed by acid hydrolysis yielded the lactones **(165a)** and **(165b)**. Mesylation followed by chromatographic separation gave the major mesylate **(166)**. The minor mesylate was converted to the lactone **(165a)** by treatment with NaOH to hydrolyse the lactone and form a transient epoxide, which lactonised in acidic conditions. This process enabled the preparation of large quantities of the major lactone **(165a)** was mesylated and then converted to the azide, which was hydrogenated to give the free amine. This was protected to afford the *N*-Boc protected lactone **(167)**, **Scheme 29**.




a) $CH_2$=CHMgBr, -30°C; b) Iva-Cl, 88%; c) LDA, THF, TMSCl, $H_3O^+$, 90%; d) DEPC, $PhCH(Me)NH_2$; e) *m*-CPBA; f) $H_3O^+$; g) MsCl, $Et_3N$; h) chromatography; i) NaOH; j) $NaN_3$, DMSO; k) $H_2$, Pd-C, EtOAc, $(Boc)_2O$.

**Scheme 29**

Jones, Nilsson and Szelke[87] reported an elegant, short stereocontrolled synthesis of the hydroxyethylene isostere Cal-ψ[CH(OTBDMS)$CH_2$]-Ala **(168)** utilising a Grignard reaction. Starting from Boc-Cal-H **(60)**, treatment with the chiral Grignard reagent **(169)** gave the alcohols **(170)** and **(171)** in the ratio 6:1. The major product **(170)** was then deprotected and reprotected with 2',2',2'-trichloro-1',1'-dimethylethoxycarbonyl (TcBoc) to give **(172)**. They used TcBoc, because it had been observed that *N*-Boc protected 3-hydroxy-4-amino acid derivatives tended to lactonise during Boc removal.[88] The alcohol **(172)** was then protected using TBDMS-OTf and oxidised with ruthenium tetroxide to yield the protected hydroxyethylene isostere **(168)**, **Scheme 30**.

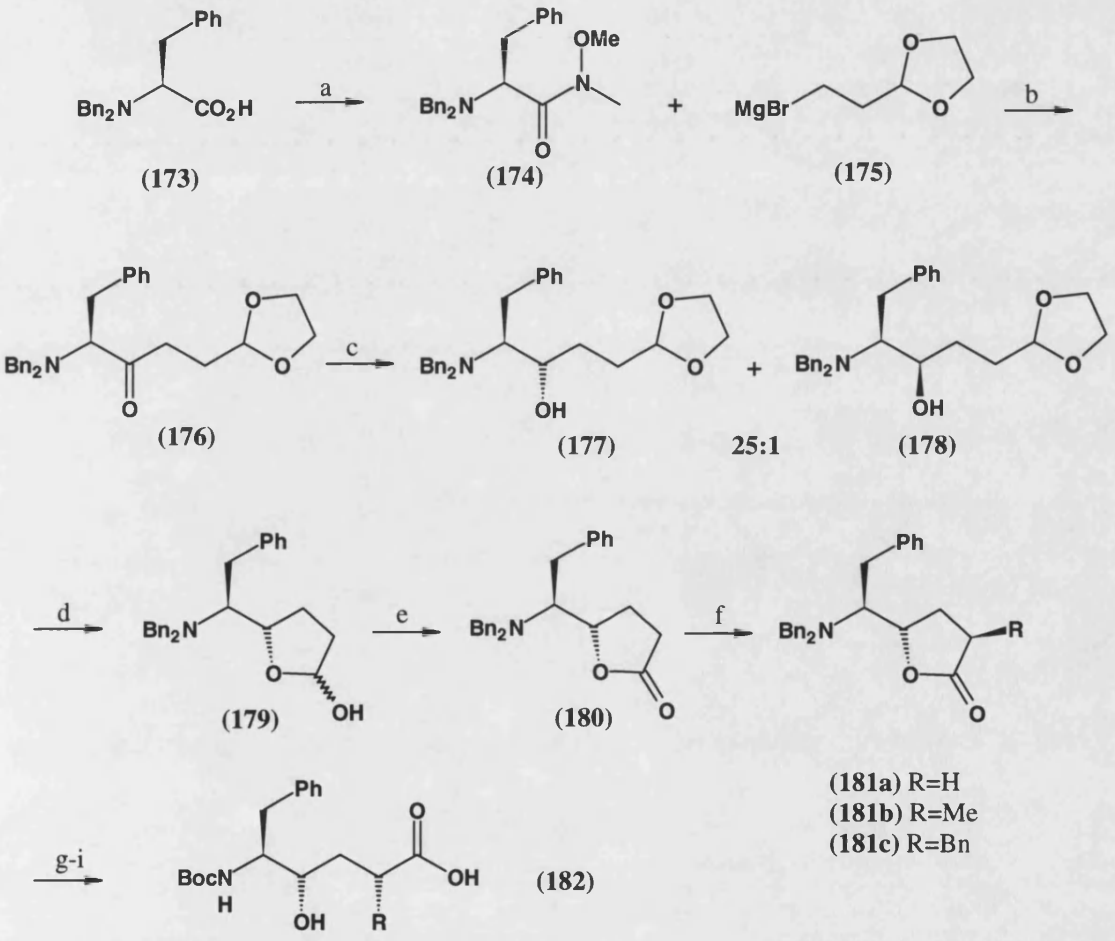



a) chromatography; b) HCl-dioxane; c) TcBoc-ONSu; d) TBDMS-OTf; e) $RuO_4$.

**Scheme 30**

Ryckman and Diederich[89] also utilised the Grignard reaction. They started from *N,N*-dibenzyl protected phenylalanine **(173)**. They prepared the *N'*-methyl-*O*-methylcarbo-xamide **(174)** using peptide coupling techniques. This was reacted with the Grignard reagent **(175)** to give the ketone **(176)**. Reduction of the ketone **(176)** using $NaBH_4$

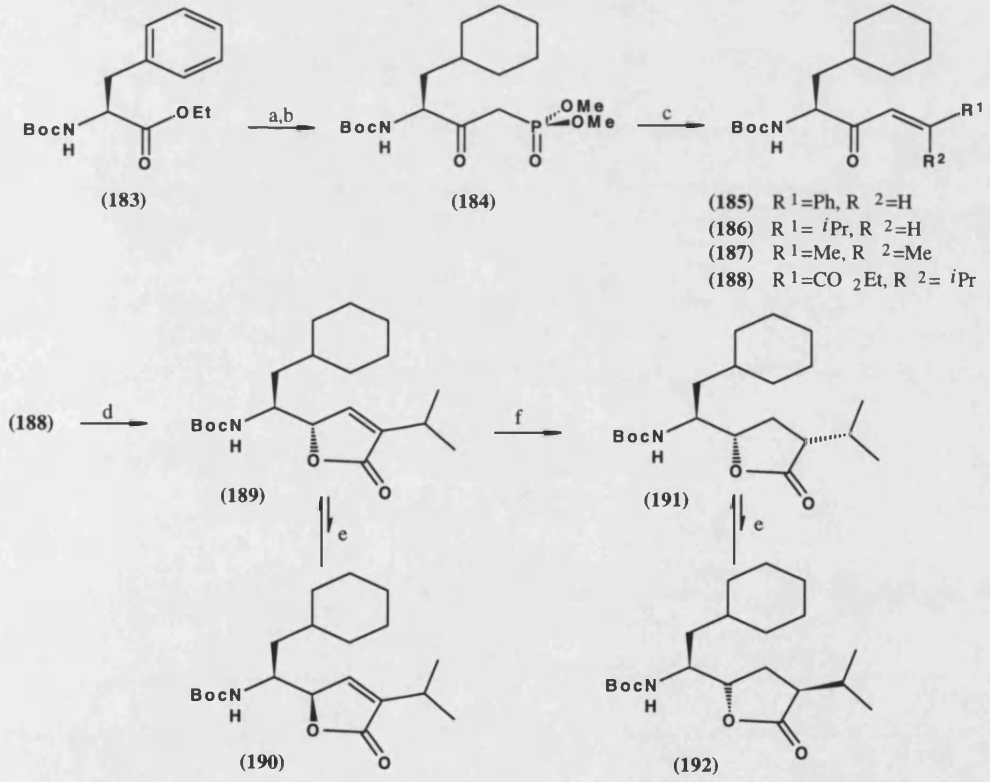
gave the alcohols **(177)** and **(178)** in good yield, alcohol **(177)** being the major product [25:1 ratio **(177)**:**(178)**]. The diastereomers were separated by chromatography and the major isomer **(177)** was lactonised to **(179)**. Oxidation gave **(180)**, which was alkylated and reprotected to give the *N*-Boc lactone **(181)**. The lactone was opened to give the desired hydroxyethylene isostere **(182)**, **Scheme 31**.




a) EDC, HOBt, HCl.NHMe(OMe), DMAP, DMF, DIPEA, RT, 95%; b) THF, 0°C, 93%; c) $NaBH_4$, MeOH, 0°C, 95%; d) 3M HCl:THF (1:1), 92%; e) $CrO_3$-$H_2SO_4$, acetone, 0°C, 95%; f) LDA, RX; g) $HCO_2H$, MeOH, Pd(black); h) $(Boc)_2O$, DMF, $Et_3N$; i) 1M NaOH, THF, citric acid, quantitative.

**Scheme 31**

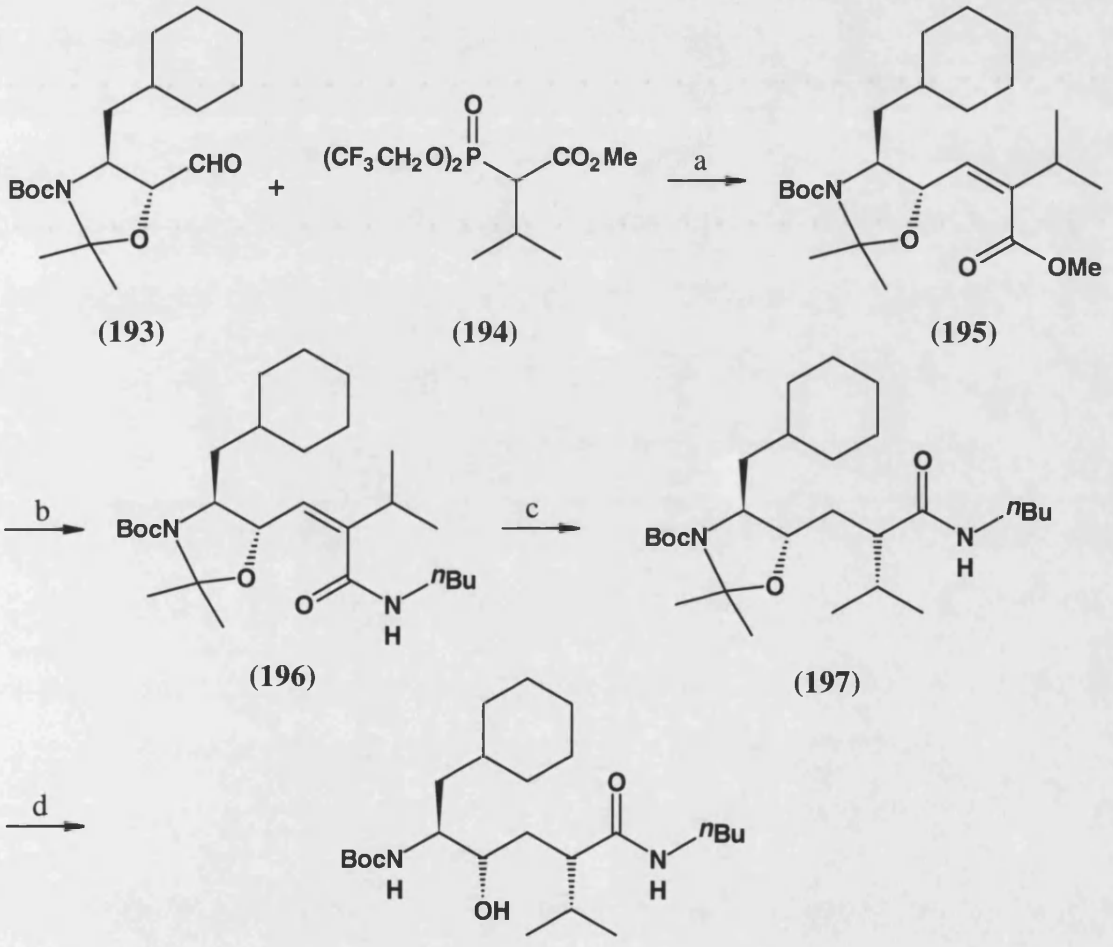
Other groups have used the Wittig reaction or modified versions to prepare hydroxyethylene isosteres. De Laszlo *et al*[90] prepared the phosphonate **(184)** from the ester **(183)**. The ester **(183)** underwent Wadsworth-Emmons reactions with benzaldehyde, *iso*-butyraldehyde and acetone to give the olefins **(185)**, **(186)** and **(187)** respectively in low yield (~30%). However, reaction with ethyl-3-methyl-2-oxobutanoate gave a single olefin **(188)** in 72-78% yield. Reduction of the olefin **(188)** gave lactones **(189)** and **(190)**. Epimerisation of these lactones with DBU / DMF at 90°C gave a 2:1 mixture of **(189):(190)**. Hydrogenation of lactone **(189)** gave the single lactone **(191)**, which was epimerised to give a 3:7 mixture of lactones **(192)** and **(191)**. Repetitive recycling of **(190)** and **(191)** provided lactone **(192)** in multi-gram quantities. Lactone **(192)** was then converted into the hydroxyethylenes by application of the Weinreb amidation reaction,[91] **Scheme 32**.




a) $H_2$, $PtO_2$, 95%; b) *n*-BuLi, $CH_3P(O)(OMe)_2$, THF, 95%; c) *n*-BuLi, ethyl-3-methyl-2-oxobutanoate or aldehyde, THF, 0°C; d) $NaBH_4$, MeOH, -30°C, (189):(190) 3:2; e) DBU, DMF, 90°C; f) $H_2$, 10% Pd/C.

**Scheme 32**

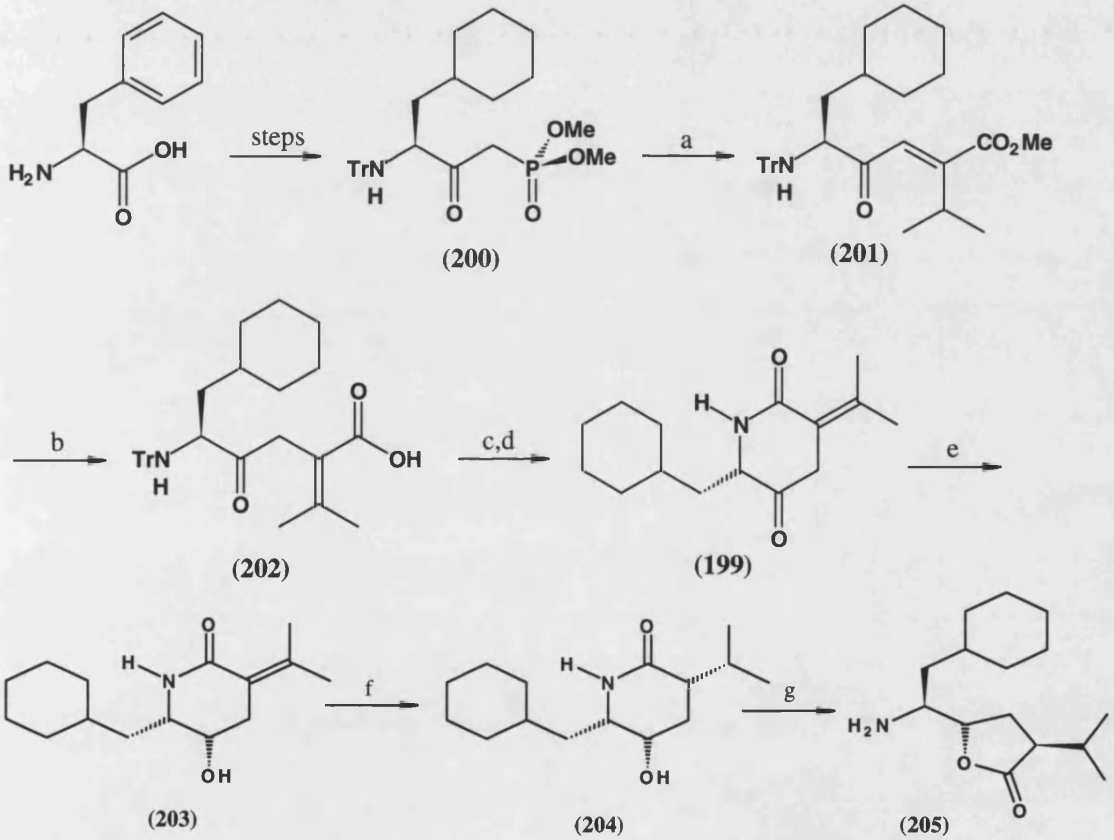
Poss and Reid[92] also used the phosphonates in their preparation. They prepared the protected aldehyde **(193)** and addition to the phosphonate **(194)** gave the α,β-unsaturated ester **(195)**. This was converted to the amide **(196)** using the Weinreb[91] amidation reaction. Hydrogenation of amide **(196)** gave predominantly diastereomer **(197)**, isolated in 70% after chromatography. The hydroxyethylene isostere **(198)** was prepared by partial hydrolysis with 10% HCl-AcOH, **Scheme 33**.




a) $KN(TMS)_2$, THF, 81%, (*Z*:*E*, 20:1), chromatography; b) *n*-$BuNH_2$, $Me_3Al$, DCE, 88%; c) $H_2$, $Rh/Al_2O_3$, THF; d) 10% HCl-AcOH, THF, 83%.

**Scheme 33**

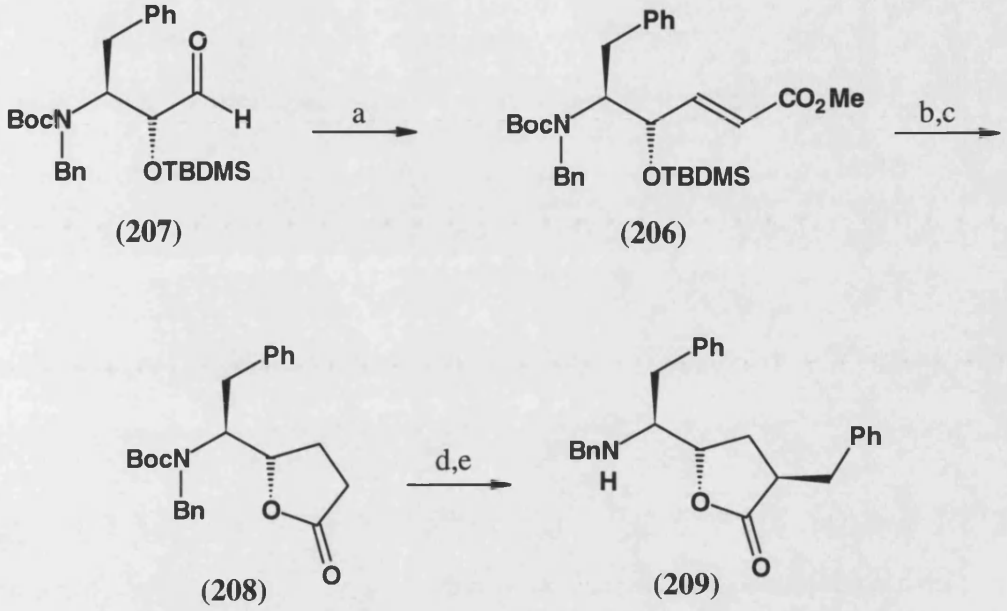
Plata, Leanna and Morton[93] also utilised a Wittig olefination. Their preparation used a novel piperidine-2,5-dione **(199)** as a chiral template. This template **(199)** was prepared from phenylalanine *via* the *N*-tritylphosphonate **(200)** following a similar procedure to de Laszlo.[90] Wadsworth-Emmons condensation gave the olefin **(201)** which, under saponification conditions, underwent double bond migration to give the thermodynamically more stable acrylic acid **(202)**. Activation of the acid, followed by acid then base conditions, afforded the chiral template **(199)**. Selective reduction with L-selectride at -78°C gave exclusively the axial alcohol **(203)**. Hydrogenation gave exclusively the δ-lactam **(204)**, which underwent lactonisation in strong acid conditions to give the hydroxyethylene isostere precursor **(205)**, **Scheme 34**.




a) i. NaH, 0°C, THF; ii. $MeO_2CCOCHMe_2$, RT, THF; b) LiOH, $H_2O$, MeOH, THF; c) *N*-hydroxysuccinimide, DCC, THF; d) i. HCl, $Et_2O$, ii. $NaHCO_3$ (aq); e) L-selectride, -78°C; f) $H_2$, Pd/C, EtOAc; g) 6M HCl, reflux.

**Scheme 34**

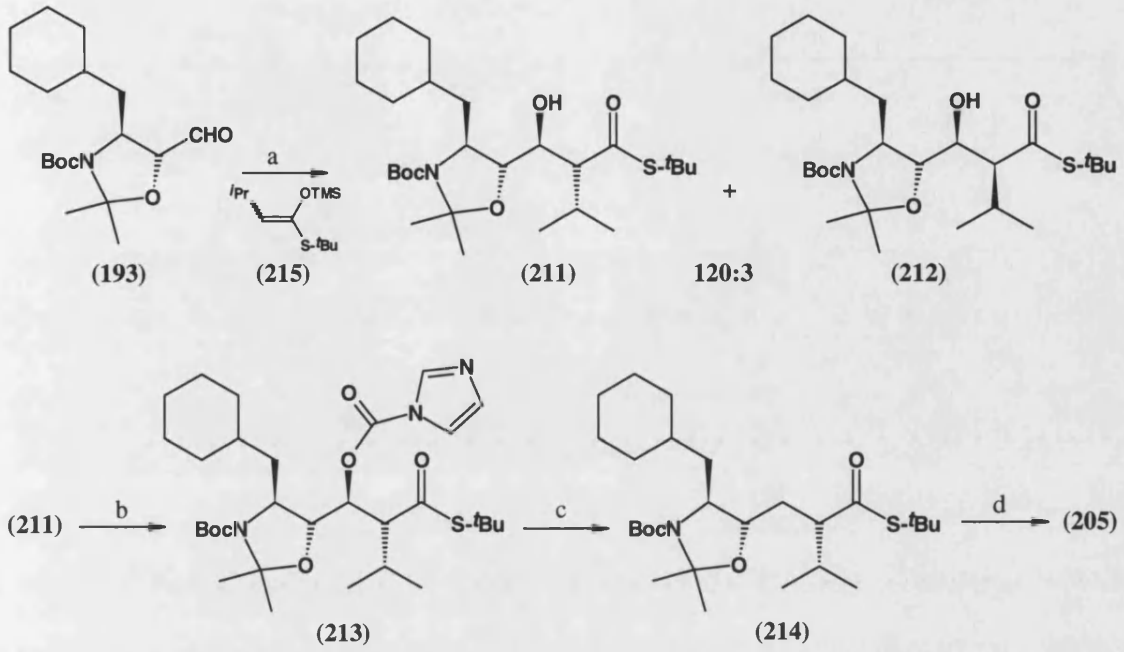
Dondoni and Perrone[94] used the stabilised phosphorus ylide, $Ph_3P{=}CHCO_2Me$, to prepare the α,β-unsaturated olefin (**206**) from *N,N*-Bn-Boc protected aldehyde (**207**). After hydrogenation of the double bond, lactonisation yielded the lactone (**208**). Benzylation and *N*-Boc removal afforded the lactone (**209**), **Scheme 35**.




a) $Ph_3P{=}CHCO_2Me$, $C_6H_6$, RT, 3 days; b) $NiCl_2.6H_2O$-$NaBH_4$, MeOH, 0°C, 1hr, RT, 18hrs; c) TBAF, $H_2O$, THF, RT, 4hrs; d) $LiN(TMS)_2$, THF, -78°C, 30mins, then BnI, -78°C, 30mins; e) 40% TFA, DCM, RT, 10mins.

**Scheme 35**

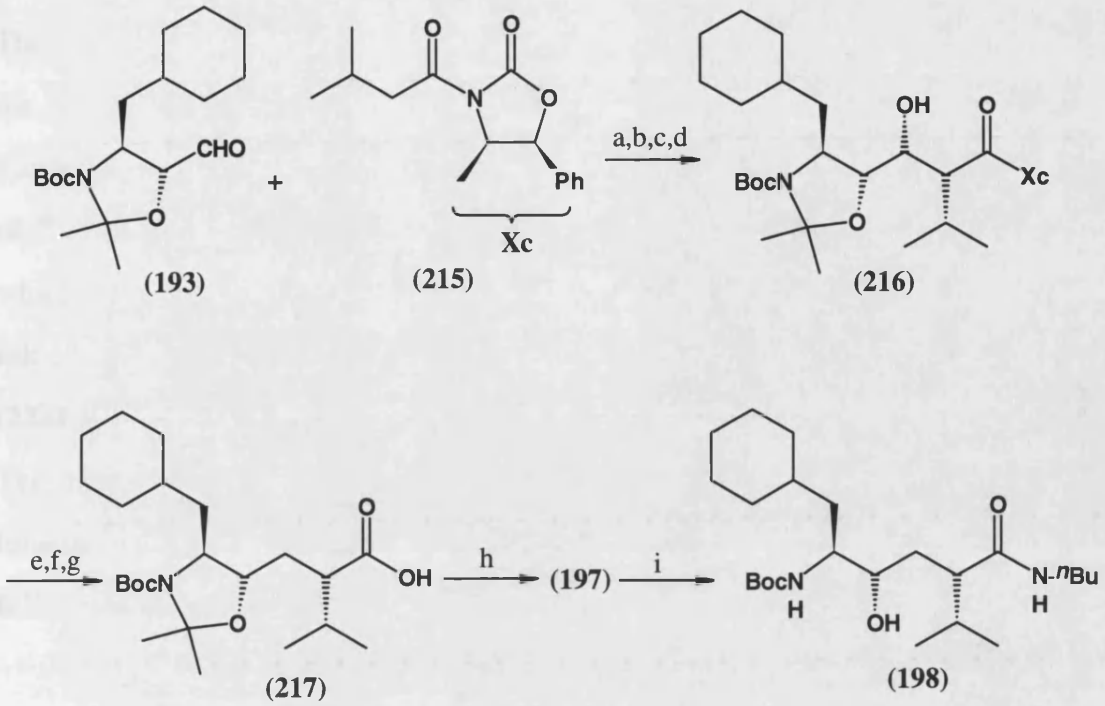
Boyd *et al*[95a] prepared hydroxyethylene isosteres from the *N*-Boc oxazolidine aldehyde (**193**). Rosenburg *et al*[95b] utilised a Lewis acid catalysed *anti*-aldol reaction, Scheme 36. They converted the aldehyde to the *anti*-aldol product (**211**) as the major product, which was separable from the minor *syn*-aldol (**212**) (1.4%). The hydroxyl moiety was removed by treating intermediate (**213**) with tributyl tin hydride to give the thio ester (**214**). Lactonisation under acid conditions gave the lactone (**205**), **Scheme 36**.




a) $BF_3.OEt_2$, DCM, -78°C, 1hr.; b) thiocarbonyldiimidazole, DMAP, DCE, 50°C, 18hrs.; c) $Bu_3SnH$, toluene, reflux, 20hrs.; d) 4M HCl, EtOH, RT, 1hr; $Na_2CO_3$.

**Scheme 36**

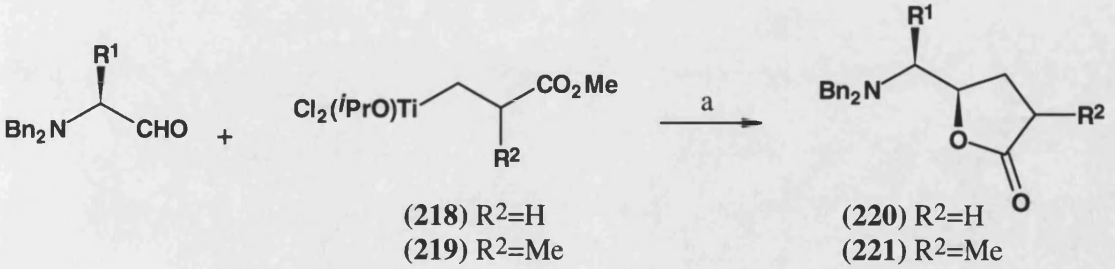
The other route Rosenburg *et al*[95b] developed used a boronyl enolate **(215)**, which they reacted with aldehyde **(193)** to yield the aldol adduct **(216)** in good yield. Removal of the hydroxyl moiety as before, and conversion to the acid **(217)** allowed for the use of standard peptide coupling techniques to give the protected hydroxyethylene isostere **(197)**. Treatment with acetyl chloride, followed by a base wash removed the N and O protecting groups to produce the target Cal-ψ[$CHOHCH_2$]-Val-NH*n*-Bu **(198)**, **Scheme 37**.




a) $n$-$Bu_2BOTf$, DCM, -78°C; b) DIPEA, -78°C, 0°C; c) (193), -78°C to 25°C; d) $H_2O_2$, MeOH, pH7, overall 63%; e) thiocarbonyldiimidazole, DMAP, DCE, 50°C, 18hrs, 84%; f) $n$-$Bu_3SnH$, toluene, reflux, 91%; g) LiOH, $H_2O_2$, THF, $H_2O$; $Na_2SO_3$; $NaHSO_4$, 90%; h) EDC, HOBt, 4-methylmorpholine, DMF, -10 to 0°C, 2 days; $BuNH_2$, 0 to 25°C, 89%; i) AcCl, MeOH, 0 to 25°C; $NaHCO_3$, 79%.

**Scheme 37**

Shibuya *et al*[96] and Decamp and Kouvaguche *et al*[97] prepared hydroxyethylene analogues by reacting α-amino aldehydes with alkoxytitanium homoenolates of the form (**218**, $R^2$=H) and (**219**, $R^2$=Me), to give the lactones (**220**, $R^2$=H), and (**221**, $R^2$=Me), **Scheme 38**.




**(218)** $R^2$=H
**(219)** $R^2$=Me

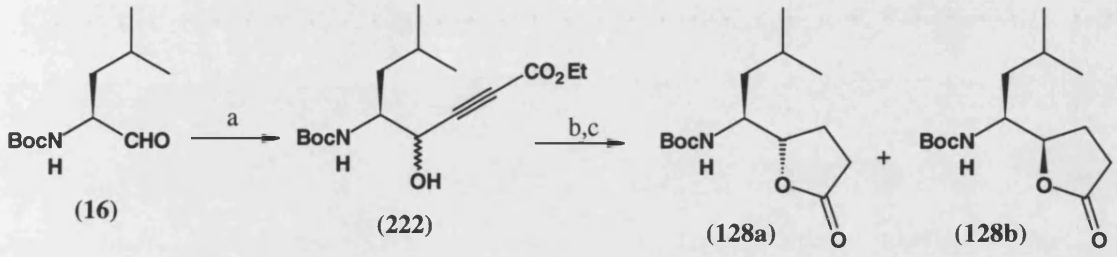
**(220)** $R^2$=H
**(221)** $R^2$=Me

a) -20°C, DCM, 12hrs.

**Scheme 38**

These precursors could then be converted to the desired hydroxyethylene isosteres using the Weinreb[91] amidation technique.

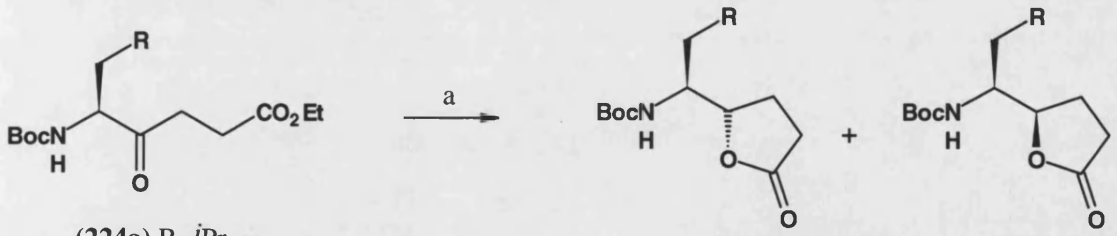
Further avenues of hydroxyethylene isostere synthesis were explored by Kleinman *et al.*[98] They developed a short, stereoselective synthesis of the lactone precursor **(128)**, which Prasad and Rich[78] prepared later. Boc-Leu-H **(16)** was reacted with the lithium salt of ethyl propiolate to generate an epimeric mixture of the hydroxy acetylenic esters **(222)** in poor yield (36%). Hydrogenation, without isomerisation, gave the keto-ester. The intermediate hydroxy esters were not isolated, but lactonised directly in refluxing toluene to yield lactones **(128)**, which were converted to the alkylated analogues following standard procedures, **Scheme 39**.




a) LiC≡CCO$_2$Et, -78°C; b) $H_2$, Pd, $BaSO_4$; c) AcOH, toluene , reflux.

**Scheme 39**

Nishi *et al*[99] also prepared the γ-lactones **(128)** and the cyclohexyl derivative **(223)** from reduction followed by lactonisation of the γ-keto ester **(224)**, **Scheme 40**.



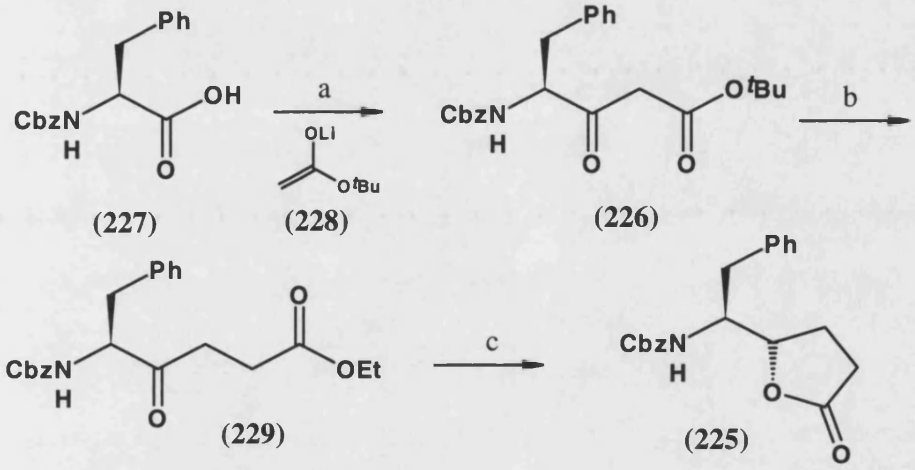

**(224a)** R=$^i$Pr
**(224b)** R=cyclohexyl

**(128a)** R=$^i$Pr **(128b)**
**(223a)** R=cyclohexyl **(223b)**

a) i. reduction; ii. cat. AcOH, toluene, reflux.

**Scheme 40**

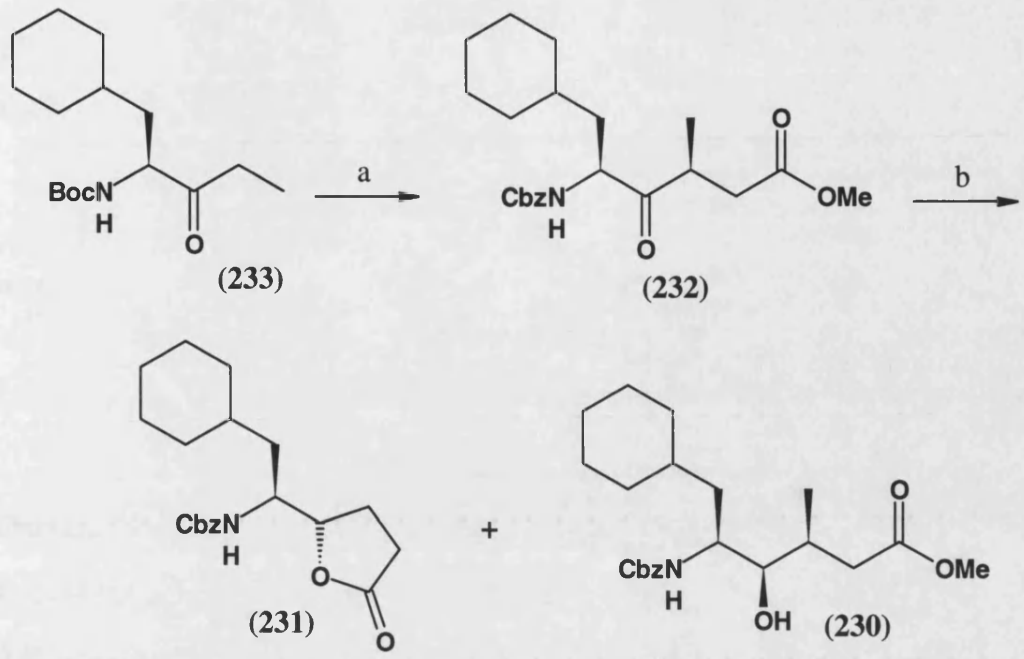
Hoffman and Kim[100] synthesised the phenylalanine γ-lactone (**225**) using a slightly modified Nishi[99] preparation. They prepared the acetylated β-keto ester (**226**) by activating Cbz-Phe (**227**) using carbonyldiimidazole (CDI) and reacting this with the enolate (**228**). The β-keto ester (**226**) was converted uneventfully to the γ-keto ester (**229**) *via* addition of the ethyl ester followed by decarboxylation. Reduction and lactonisation gave the γ-lactone (**224**), **Scheme 41**.




a) CDI, (228); b) i. NaH; ii. $BrCH_2CO_2Et$; iii. TFA, 76%; c) i. $NaBH_4$; ii. AcOH, toluene, reflux.

**Scheme 41**

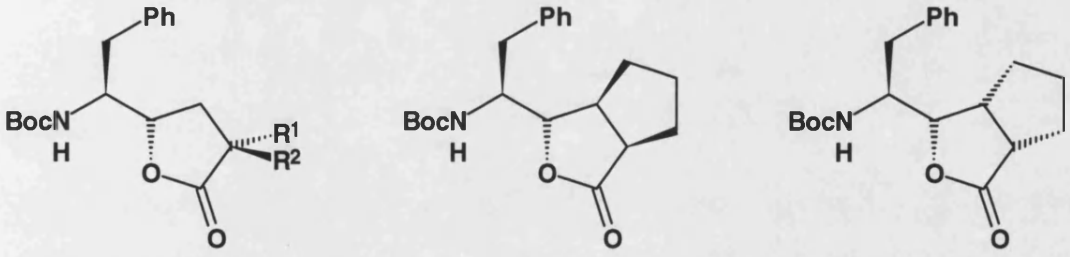
Randuz *et al*[101] prepared the hydroxyethylene isostere (**230**) and the lactone (**231**) from reduction of the keto ester (**232**). The ester was prepared *via* enolisation of the ketone (**233**), which was trapped with methylbromoacetate. The keto ester (**232**) was the only diastereomer isolated. The ketone (**233**) was prepared in large scale following the procedure of Rapport,[102] **Scheme 42**.




a) LDA, THF, -78°C, methylbromoacetate; b) $LiBH_4$, 2-propanol, -50°C, 30mins.

**Scheme 42**

Sakuri *et al*[103] prepared many γ-lactone precursors of hydroxyethylene isosteres following a similar procedure to that of Nishi,[99] **Figure 10**.



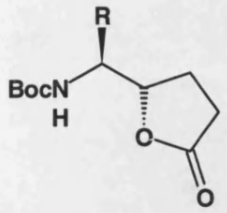

**(234)** $R^1$=H, $R^2$=Me
**(235)** $R^1$=Me, $R^2$=H

**(236)**

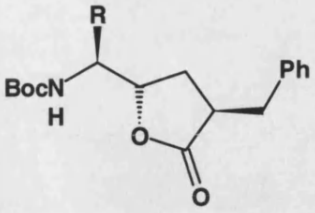
**(237)**

**Figure 10**

Lagu and Liotta[104] also followed a similar synthesis to that of Nishi[99] and isolated the γ -lactone precursors **(238)** and **(239)**, **Figure 11**.




**(238a)** R=Me
**(238b)** R=Bn

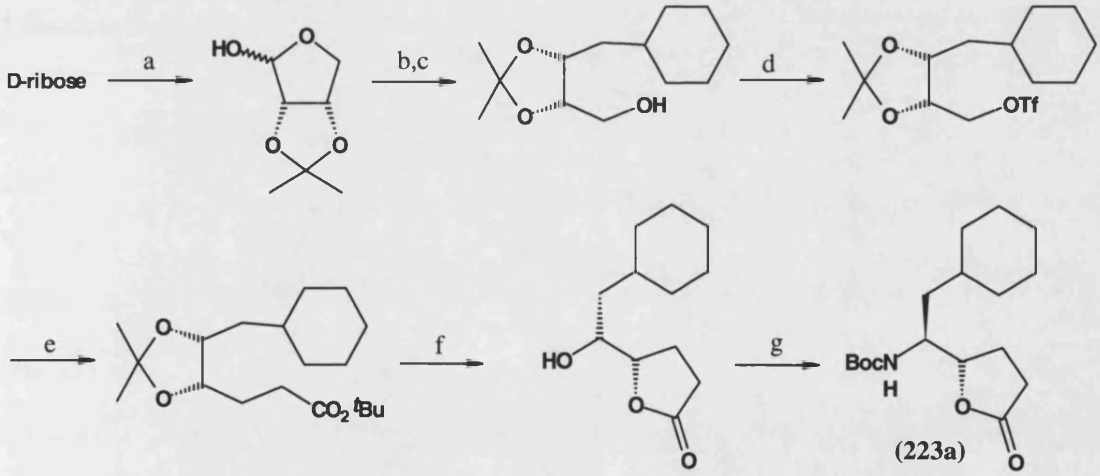



**(239a)** R=Me
**(239b)** R=Bn

**Figure 11**

Shibuya *et al*[105] reviewed some of the previous literature in 1992, which incorporated the synthesis of hydroxyethylene isosteres from β-oxygenated g-amino acids and γ-oxygenated δ-amino acids.

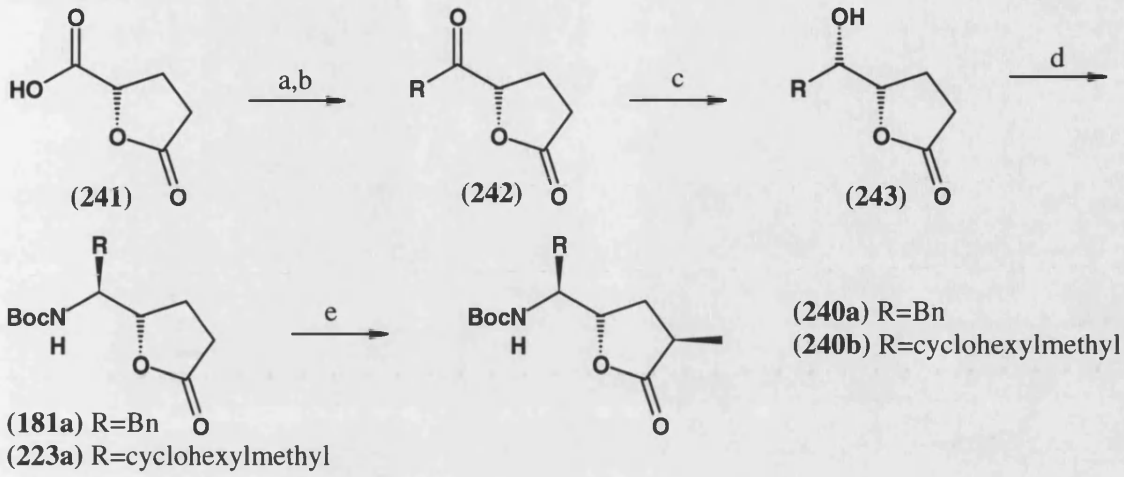
Kotsuki *et al*[106] and Melovi *et al*[107] prepared hydroxyethylene isosteres from D-ribose and D-isoascorbic acid respectively. **Scheme 43** shows the chemical transformations used to prepare the γ-lactone **(223a)**.




a) i. $H_2SO_4$, acetone, RT, 0.5hr.; ii. $NaBH_4$, 0°C, 2hrs; iii. $NaIO_4$, RT, 2hrs.; b) $Ph_3P$-cyclohexylylide, THF, -30°C; c) $H_2$, Raney Ni, EtOH; d) triflic anhydride, pyridine, -15°C, 15mins.; e) $LiCH_2CO_2{}^tBu$, THF, DMPU, -78°C, 5mins; f) TFA, RT, 1hr.; g) i. MsCl, pyridine, ii. $NaN_3$, 18-crown-6, DMPU, 80°C, 36hrs; iii. $H_2$, 10% Pd/C, $Boc_2O$, EtOAc.

**Scheme 43**

Nishi *et al*[108] prepared γ-lactone precursor **(240)** from 5-oxotetrahydrofuran-2-carboxylic acid **(241)**, **Scheme 44**.



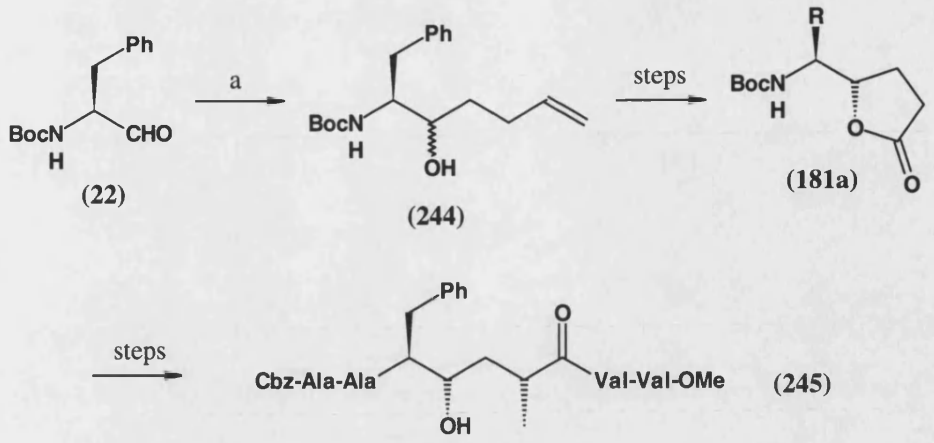

a) $SOCl_2$, reflux; b) cyclohexyl-$CH_2MgBr$ or $PhCH_2MgCl$, THF, -78°C; c) L-Selectride, THF, -78°C, d) i. MsCl, $Et_3N$, DCM, 0°C; ii. LiBr, THF, reflux; iii. $NaN_3$, DMPU, RT; iv. $H_2$, Pd/C, $Boc_2O$, EtOAc, RT; e) LDA, THF, -78°C; MeI.

**Scheme 44**

### 1.4.2.2 HIV-1 inhibitors

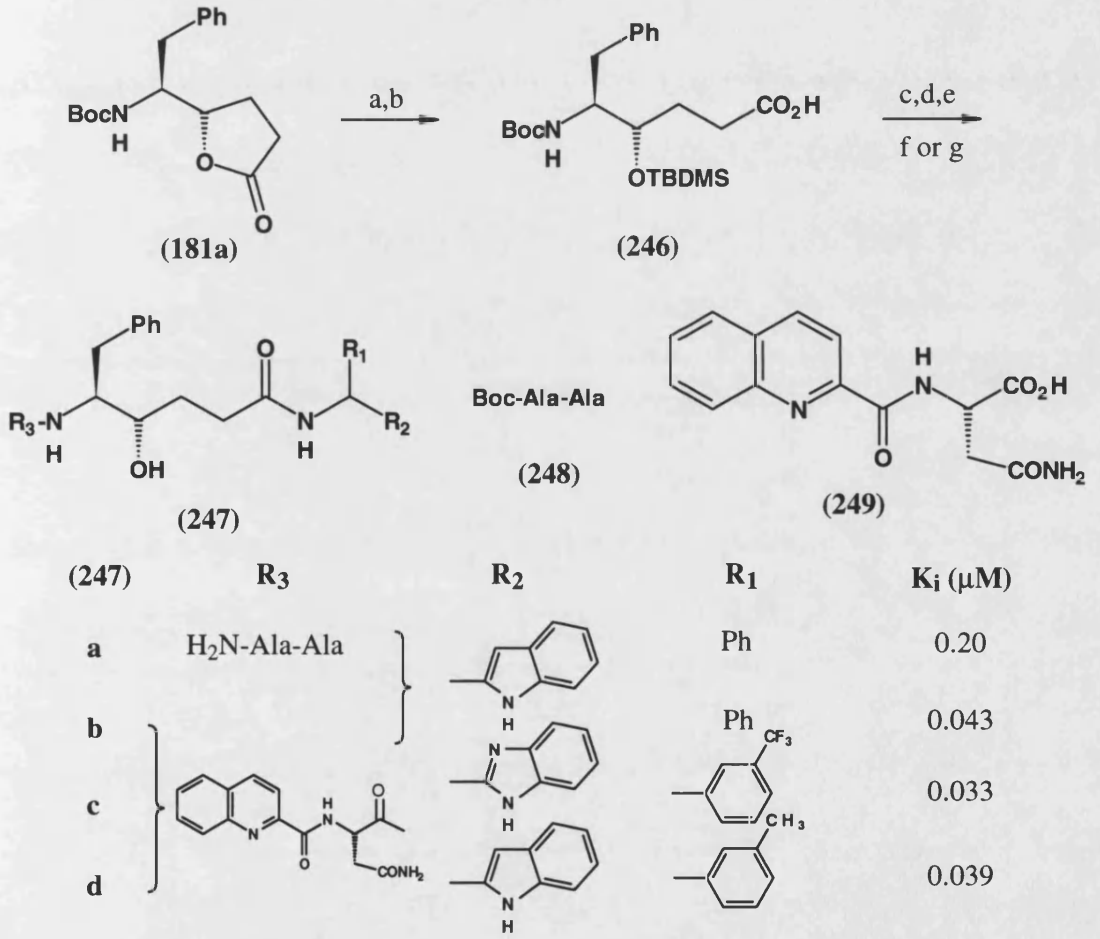
Most of the HIV-1 hydroxyethylene isostere inhibitors synthesised since the start of this decade have involved the use of the γ-lactones **(181a)** and **(236)**. Dreyer *et al*[109] and Varney *et al*[110] both prepared their isosteres from the γ-lactone precursor **(181a)**. Dreyer utilised a Grignard condensation to prepare the γ-hydroxyalkene **(244)** from Boc-Phe-H **(22)**. Following a similar procedure to that of Prasad and Rich,[78] they prepared the γ-lactone precursor **(181a)**, which was transformed to the hydroxyethylene analogue **(245)**, **Scheme 45**.

Varney *et al*[110] converted the γ-lactone **(181a)** to the protected γ-hydroxy acid **(246)**, this was then converted to a number of HIV-1 protease inhibitors **(247)**, **Scheme 46**, of which **(247c)** was the most potent inhibitor.




a) $BrMg(CH_2)_2CHCH_2$, ether, toluene.

**Scheme 45**

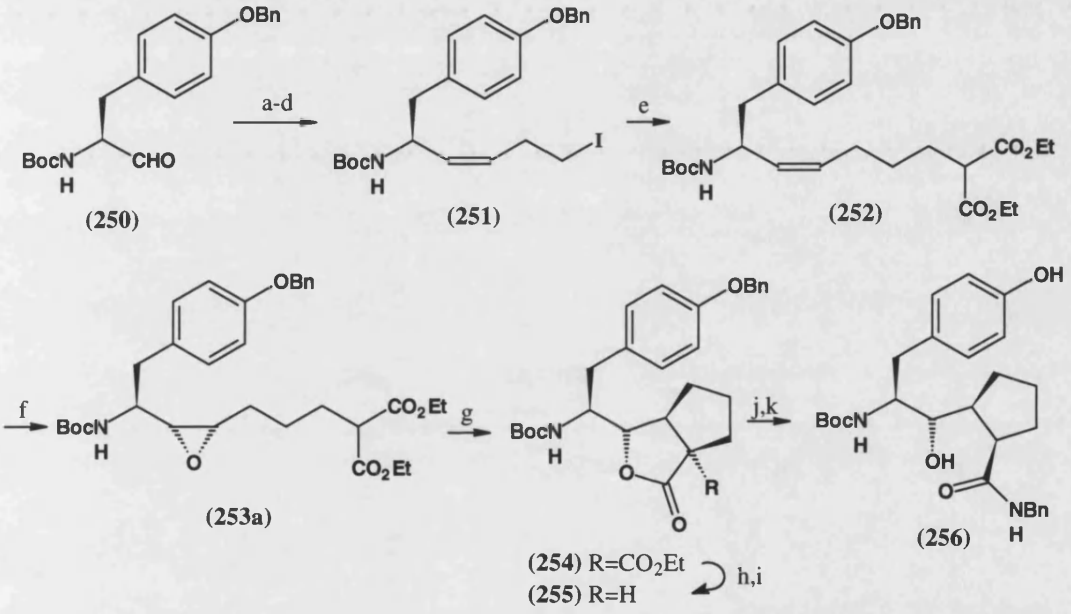



| (247) | $R_3$ | $R_2$ | $R_1$ | $K_i$ (μM) |
|---|---|---|---|---|
| a | $H_2N$-Ala-Ala |  | Ph | 0.20 |
| b | |  | Ph  | 0.043 |
| c |  | |  | 0.033 |
| d | |  |  | 0.039 |

a) 2M LiOH, THF; b) TBDMSCl, imidazole, DMF; c) DCC, amine, DCM; d) TFA, DCM; e) TBAF, THF; f) i. DCC, acid (**248**); DMF; ii. TFA, DCM; or g) DCC, acid (**249**), DMF, HOBt, $Et_3N$.

**Scheme 46**

Thompson *et al*[111], Cushman *et al*[112] and Sakurai *et al*[113] all prepared derivatives of the γ-lactone (**236**). Thompson *et al*[111] prepared this intermediate using a Wittig reaction to give the *cis*-alkene from the *N*-Boc aminoaldehyde (**250**). Conversion to the iodoalkene (**251**) allowed the formation of the diester (**252**). Epoxidation gave mainly the *threo* epoxide (**253a**) which was lactonised to the bicyclic lactone (**254**). Decarboxylation yielded the γ-lactone (**255**), which was finally converted to the hydroxyethylene analogue (**256**), **Scheme 47**.

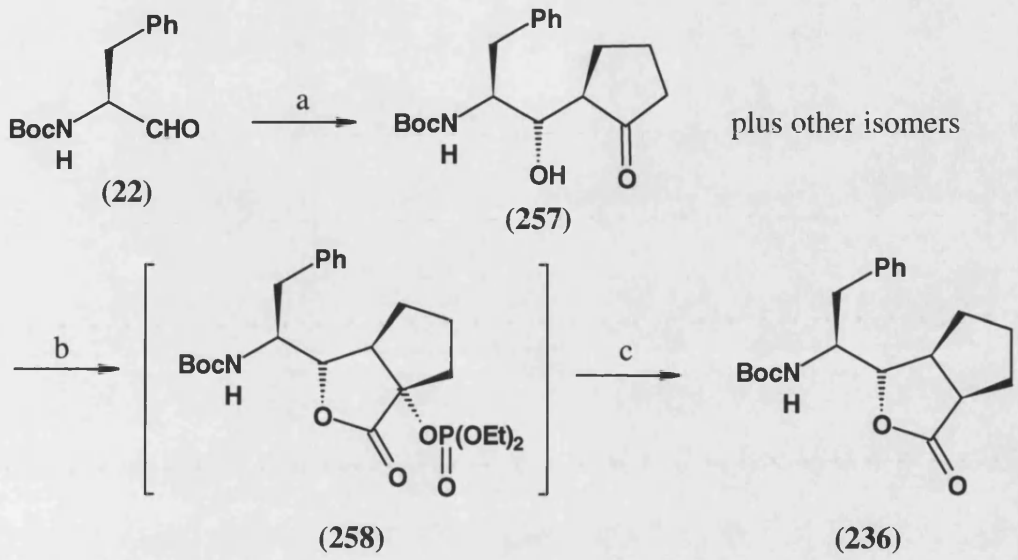



a) $Ph_3P^+(CH_2)_3CO_2H\ Cl^-$, $KN(TMS)_2$, THF, 0°C to 25°C, 18 hrs; b) $Et_3N$, EtOCOCl, THF, -10°C to -2°C, 45 mins., then $NaBH_4$, MeOH, 0°C, 1 hr; c) $MeSO_2Cl$, $Et_3N$, 0°C; d) NaI, acetone, 56°C, 3 hrs; e) NaH, $CH_2(CO_2Et)_2$, DMF, 80°C, 45 mins., f) *m*-CPBA, DCM, -10°C to 25°C, 4 hrs; g) $LiN(TMS)_2$, then $ZnCl_2$, THF, $Et_2O$, -20°C to 25°C, 3 days; h) LiOH, dioxane, $H_2O$, 25°C, 6 hrs; i) xylenes, 160°C, 10 hrs; j) $PhCH_2NH_2$, *n*-BuLi, THF, -78°C, 1½ hrs; k) 5% Ph/C, EtOH, THF, $H_2$, 20 hrs.

**Scheme 47**

Cushman *et al*[112] showed that the γ-lactone (**236**) could be generated from Boc-Phe-H (**22**) *via* an aldol condensation with cyclopentanone, giving four diastereomers. Treatment of the β-hydroxyketone (**257**) with LiCN and DEPC gave the intermediate

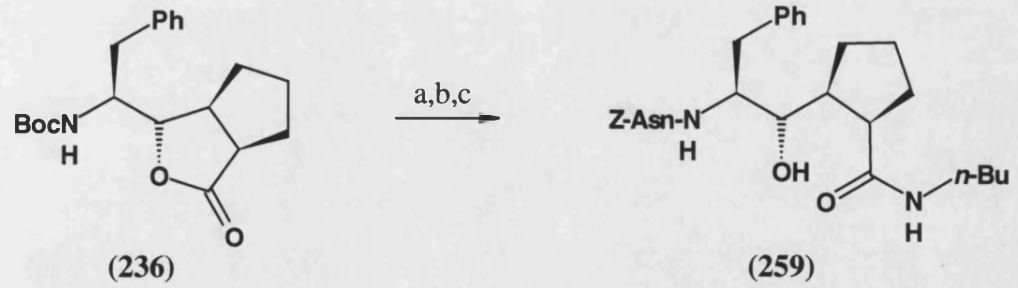
(258) which upon reaction with samarium iodide yielded the γ-lactone (236), **Scheme 48**.




a) cyclopentanone, LDA, THF, -78°C, 1½ hrs.; b) LiCN, DEPC, THF, 0°C, 20 mins; c) $SmI_2$.

**Scheme 48**

Sakurai *et al*[113] also used the γ-lactone (236). This was incorporated into the hydroxyethylene peptidomimetic (259), **Scheme 49**.

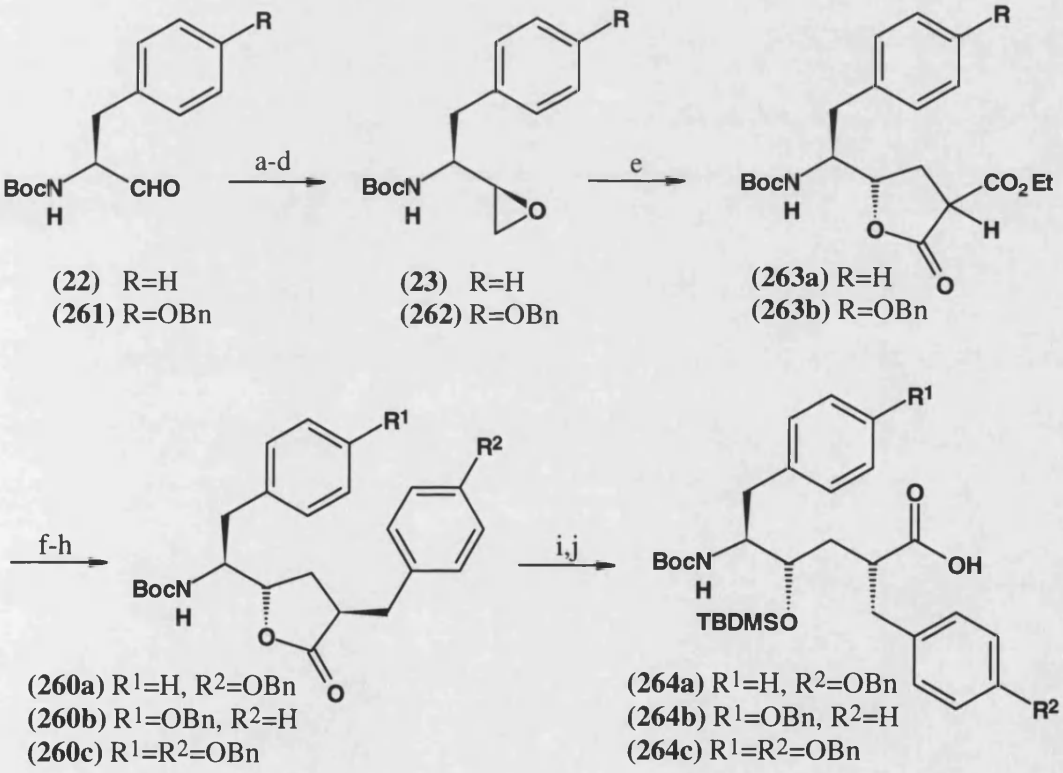



a) 4M HCl, dioxane; b) Z-Asn-*O*-nitrophenyl, $Et_3N$; and c) *n*-$BuNH_2$.

**Scheme 49**

W.J. Thompson *et al*[114], Askin *et al*[115] and S.K. Thompson *et al*[116] utilised the γ-lactone precursor **(260)** to prepare HIV-1 inhibitors. W.J. Thompson *et al*[114] prepared the γ-lactone **(260)** from the *N*-Boc aminoaldehyde **(261)** and **(22)**. Peterson reaction, followed by reprotection and then epoxidation gave the *threo* epoxide **(262)** and **(23)** as the major isomer. Lactonisation gave γ-lactone esters **(263)**, followed by decarboxylation to the γ-lactones **(260)**. These were converted to the protected hydroxyethylene peptidomimetics **(264)**.

Deprotection and amidation gave the targets **(265)**. Finally, hydrogenation gave the corresponding phenol derivatives **(266)**, **Scheme 50**.



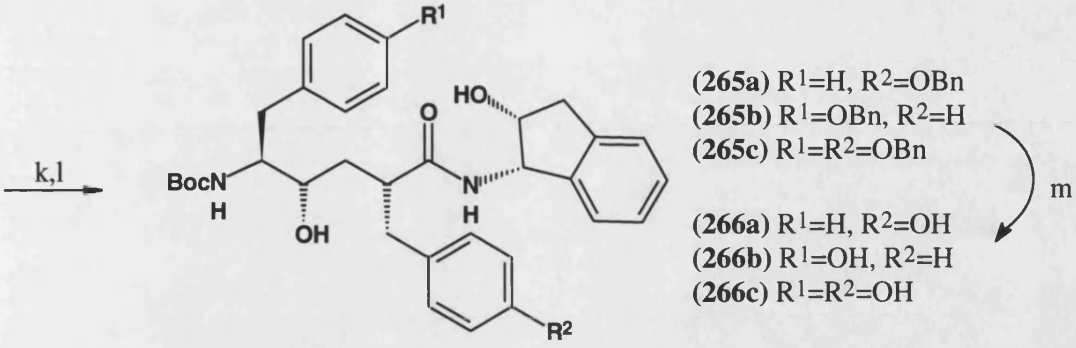

**(22)** R=H
**(261)** R=OBn

**(23)** R=H
**(262)** R=OBn

**(263a)** R=H
**(263b)** R=OBn

**(260a)** $R^1$=H, $R^2$=OBn
**(260b)** $R^1$=OBn, $R^2$=H
**(260c)** $R^1$=$R^2$=OBn

**(264a)** $R^1$=H, $R^2$=OBn
**(264b)** $R^1$=OBn, $R^2$=H
**(264c)** $R^1$=$R^2$=OBn




a) $TMSCH_2MgCl$; b) $BF_3.OEt_2$, DCM; c) $Boc_2O$; d) *m*-CPBA, DCM or magnesium monophthalate hexahydrate, MeOH; e) $CH_2(CO_2Et)_2$, Na/EtOH; f) BnBr or 4-$(PhCH_2OC_6H_4)CH_2Cl$, Na/EtOH; g) LiOH; h) toluene, reflux, followed by chromatography; i) LiOH; j) TBDMSCl, imidazole, DMF, MeOH; k) 1(*S*)-amino-2(*R*)-hydroxyindane, HOBt, EDC, DMF; l) TBAF, THF; m) $H_2$, 10% Ph/C, THF, MeOH.

**Scheme 50**

Askin *et al*[115] also prepared inhibitors (**268**) similar to those of W.J. Thompson *et al*[114] from an intermediate γ-lactone (**267**). The inhibitors they prepared are shown in **Figure 12**.

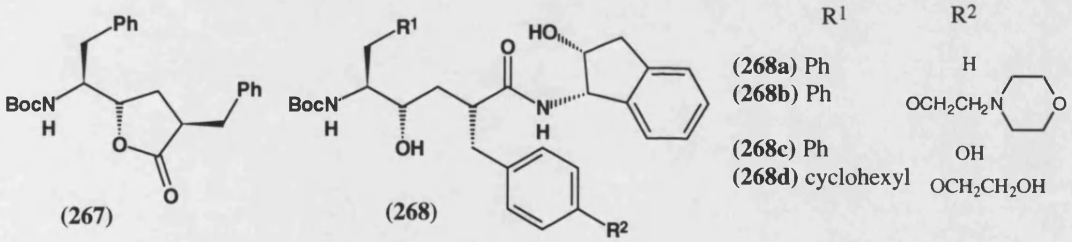
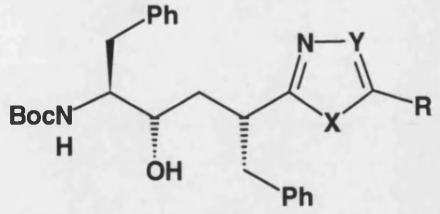



**Figure 12**

S.K. Thompson *et al*[116] converted the γ-lactone (**267**) into a variety of inhibitors (**269**) which contained the hydroxyethylene unit, **Figure 13**.

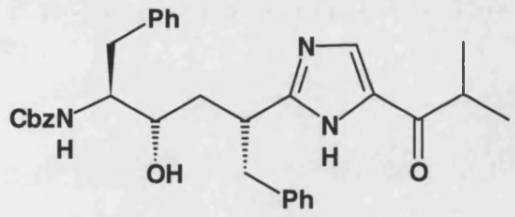



X=NH, S
Y=CH, N
R=alkyl

**(269)**

**Figure 13**

S.K. Thompson *et al*[117] used the protected hydroxyethylene analogue **(29)** which Evans *et al*[62] had prepared in 1985, for the preparation of numerous HIV-1 inhibitors, the most active being the inhibitor **(270)**, **Figure 14**.




$K_i$

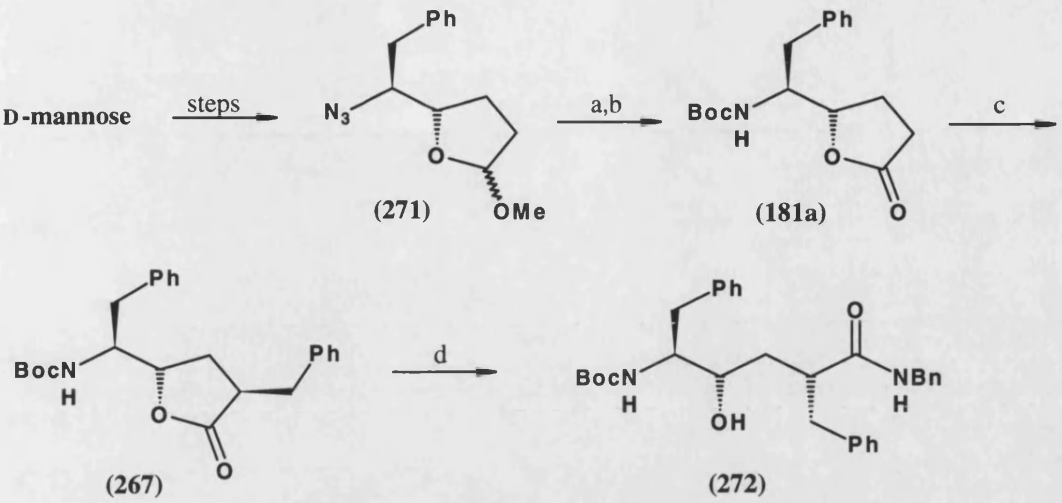
0.6 nM

**(270)**

**Figure 14**

Chu *et al*[118] and Chakraborty *et al*[119] derived hydroxyethylene analogues from the naturally occurring optically active sugars, D-mannose and D-glucose, respectively.

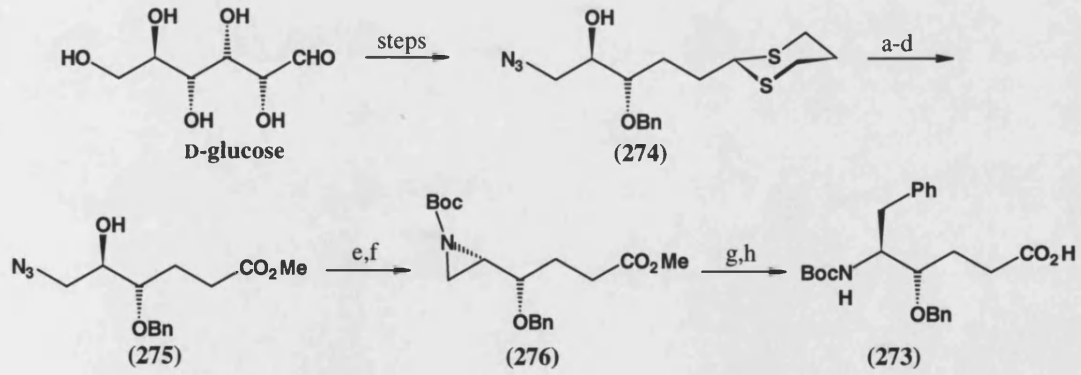
Chu *et al*[118] converted D-mannose to the cyclic ether **(271)** in nine steps. The ether subjected to by Grieco oxidation,[120] followed by hydrogenation in the presence of di-*tert*-butyl dicarbonate ($Boc_2O$) to give the γ-lactone **(181a)**. Standard alkylation yielded the γ-lactone **(267)** which was converted to the hydroxyethylene analogue **(272)** using Weinreb[91] amidation, **Scheme 51**.




a) *m*-CPBA, $BF_3.OEt_2$, DCM, $0^oC$, 3 hrs., b) 10%, Pd/C, $H_2$ EtOAc, $Boc_2O$, 6 hrs; c) $LiN(TMS)_2$, THF, $-78^oC$, 30 mins.; $PhCH_2I$, $-78^oC$, 30 mins, then $MeCH_2CO_2H$, $-78^oC$ to $23^oC$, 15 mins.; d) $Me_3Al$, $PhCH_2NH_2$, DCM, $40^oC$, 3 hrs.

**Scheme 51**

Chakraborty *et al*[119] described an elegant and concise method for the preparation of the hydroxyethylene peptidomimetic **(273)** from D-glucose. The sugar was converted to the azido alcohol **(274)** in several steps. Functional group manipulations gave the azido alcohol **(275)** which was converted to the aziridine **(276)** before opening to give the benzyl hydroxyethylene analogue **(273)**, **Scheme 52**.



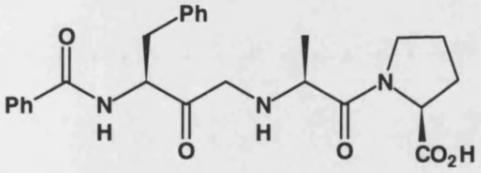

a) $Ac_2O$, pyridine, 12 hrs; b) HgO, $BF_3.OEt_2$, 85% aq THF, RT, 20 mins; c) PDC, DMF, RT, 4 hrs, then $CH_2N_2$; d) $K_2CO_3$, MeOH, 1 hr; e) $Ph_3P$, $C_6H_6$, 1 hr., reflux, $(Boc)_2O$, RT; or step e. followed by f) $Ph_3P$, DEAD, RT, 1 hr; g) PhMgBr, CuBr, DMS, toluene, $-30^oC$, 1 hr; h) 1M LiOH, THF, RT, 6 hrs.

**Scheme 52**

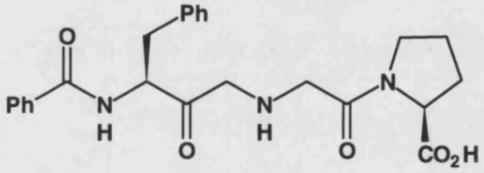
Martin,[121] Huff[122] and Marshall[123] have recently reviewed the preparation of hydroxyethylene and hydroxyethylamine isosteres for the inhibition of HIV protease.

### 1.4.3 Hydroxyethylamine isosteres

Gordon *et al*[124] developed a new class of ACE inhibitors in the mid-80's. This new class of inhibitor contained the ketomethyl unit instead of the amide bond. Substances of this type were reported to be the most potent at the time with $IC_{50}$ of 3nM for inhibitor **(277)**,[124a] **Figure 15**.



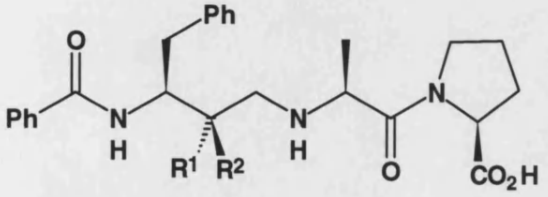
**(277)**



**(278)**

**Figure 15**

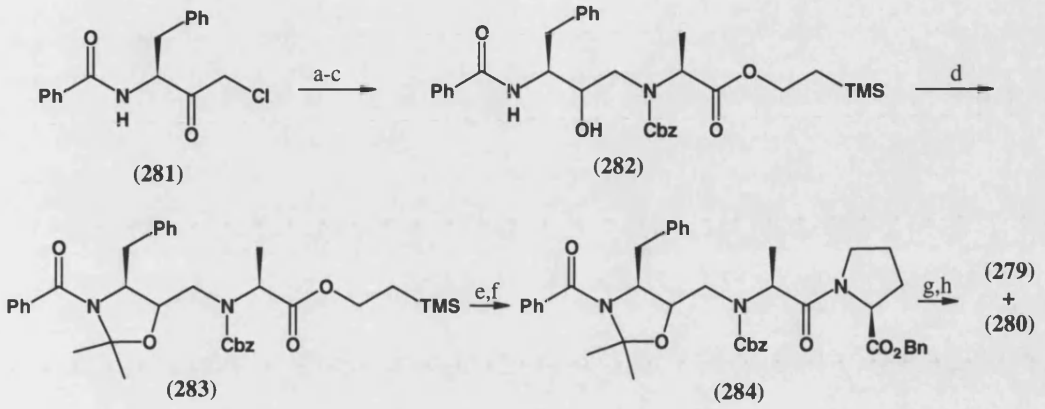
Modification of the backbone produced more potent inhibitors with **(278)**[124b] being the most active inhibitor with $IC_{50}$ of 0.004 nM. In 1985, Gordon *et al*[124c] incorporated the aminoalcohol unit, on the hypothesis that this modification of the amide bond at the scissile site may mimic the putative transition state, an idea very similar to that considered for the hydroxyethylene analogue. They prepared the first hydroxyethylamine peptidomimetic **(279)** and found the alcohol with the (*R*) configuration to be 300 times more active than the corresponding (*S*) isomer **(280)**.



| $IC_{50}$ (nM) | | R1 | R2 |
|---|---|---|---|
| 28 | **(279)** | OH | H |
| 10,000 | **(280)** | H | OH |

Gordon *et al*[124d] prepared the inhibitor **(279)** from the *N*-benzamide chloroketone **(281)**. This was coupled with Ala-$OCH_2CH_2TMS$. Protection of the secondary

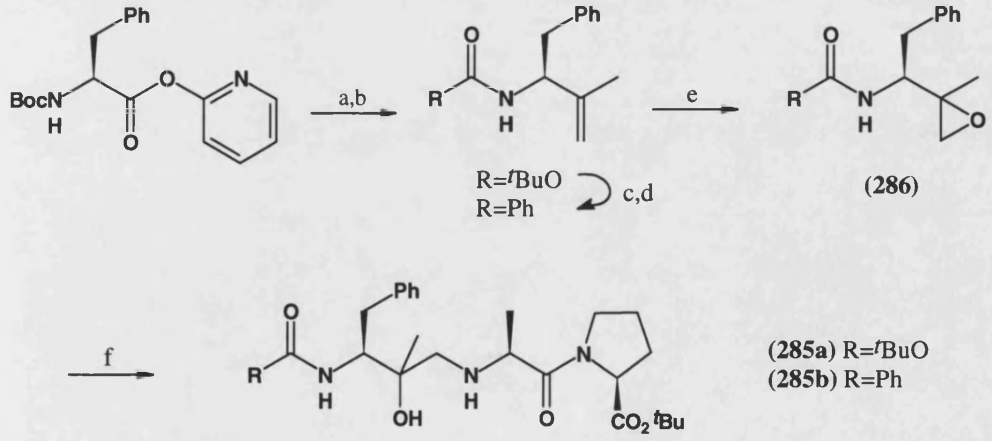
amine followed by reduction of the ketone with sodium borohydride gave the alcohol **(282)**. Protection of the alcohol gave **(283)**, deprotection and coupling gave the protected hydroxyethylamine **(284)**. This was deprotected to furnish the inhibitors **(279)** and **(280)**, **Scheme 53**.




a) Ala-OC$(CH_2)_2$TMS, DMF, NaI, $NaHCO_3$; b) Cbz-Cl, $C_6H_6$, pyridine; c) $NaBH_4$, THF, water; d) 2-methoxypropene, pyridinium *para*-toluene sulfonic acid; e) TBAF, DMF; f) 2-morpholinoethylisocyanide, HOBt, Pro-OBn, THF; g) THF, AcOH, 10%, HCl; (6:4:4), 25°C; h) $H_2$, Pd/C, HCl, EtOH.

**Scheme 53**

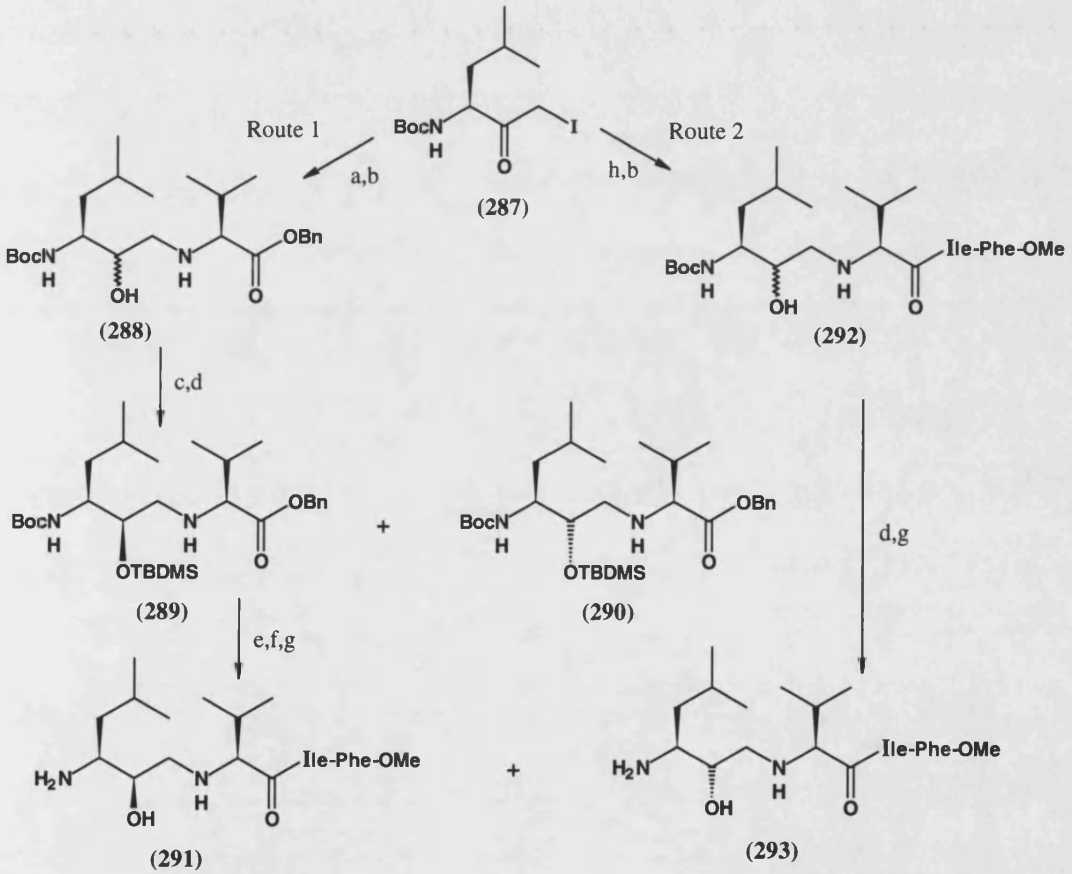
Gordon *et al*[124e] also prepared the 3° alcohol **(285)** to examine the constraints on enzyme binding. This synthesis involved the use of the epoxide **(286)** which was ring-opened to yield the tertiary analogue **(285)**, **Scheme 54**.




a. $CH_3MgBr$, THF, -78°C; b. $CH_3PPh_3Br$, $KN(TMS)_2$, $C_6H_6$; c. TFA, DCM; d. PhCOCl, DIPEA; e. *m*-CPBA, DCM; f. Ala-Pro-O$^t$Bu, MeOH, 65°C.

**Scheme 54**

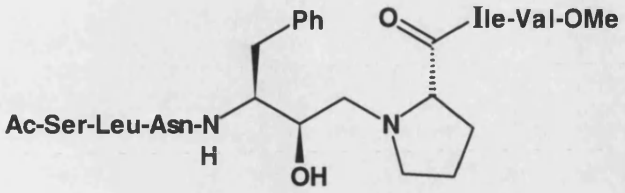
Harris *et al*[125] also saw the potential of this new class of inhibitor and developed Leu-Val bond mimetics that contained the hydroxyethylamine unit. They prepared their analogues from the iodo ketone (**287**) following two routes, **Scheme 55**. The first route involved the coupling with Val-OBn followed by reduction, to give the dipeptide mimetic (**288**), this was protected and then the (*R*) and (*S*) isomers (**289**) and (**290**) were separated by chromatography. Hydrogenation and then coupling gave the inhibitor (**291**). Route two involved coupling of (**287**) with Val-Ile-Phe-OMe, then reduction to give the alcohol (**292**). The isomers were then separated and the Boc group removed to give both isomers (**291**) and (**293**).




a) Val-OBn, THF, 40°C; b) $NaBH_4$, MeOH; c) TBDMSCl, DMAP, DMF, $Et_3N$; d) chromatography; e) $H_2$, Pd; f) DIPEA, HCl.Ile-Phe-OMe, HOBt, EDC; g) HCl, MeOH; h) Val-Ile-Phe-OMe, THF, 40°C.

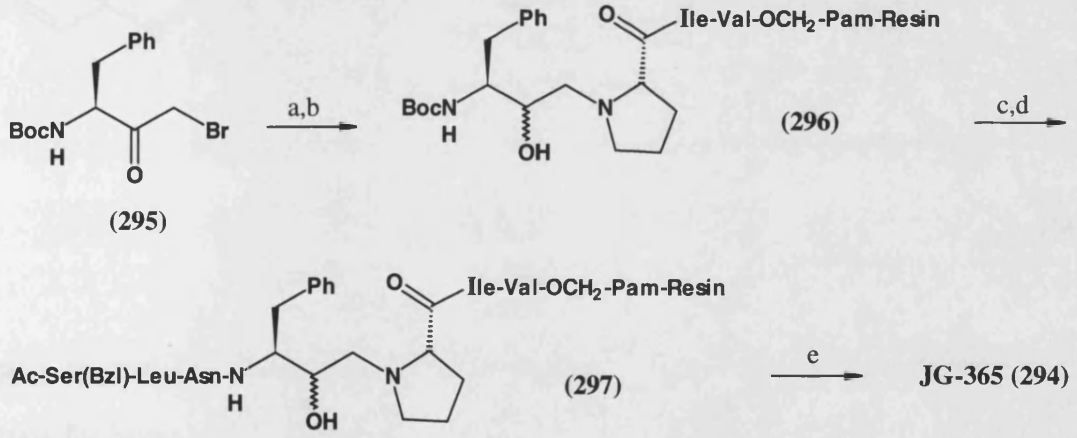
**Scheme 55**

Rich and Kent *et al*[126] showed that the JG-365 (**294**) was an active HIV-1 inhibitor ($K_i$ of 0.24nM). JG-365 (**294**) was shown to be 3000 more potent than the reduced amide analogue MVT-101 (*N*-Ac-Thr-Ile-Nle-ψ[$CH_2NH$]-Nle-Gln-Arg-$NH_2$).




**JG-365 (294)**

Alewood and Kent *et al*[127] utilised solid phase chemistry to prepare this analogue (**294**), **Scheme 56**. Starting from the bromo ketone (**295**) coupling with TFA-Pro-Ile-Val-$OCH_2$-Pam-Resin ($OCH_2$-Pam = 4-(carboxamidemethyl)benzylester), followed by reduction gave the alcohol (**296**). This was then converted to heptapeptide mimetic (**297**). Removal from the resin and deprotection gave the target inhibitor JG-365 (**294**).



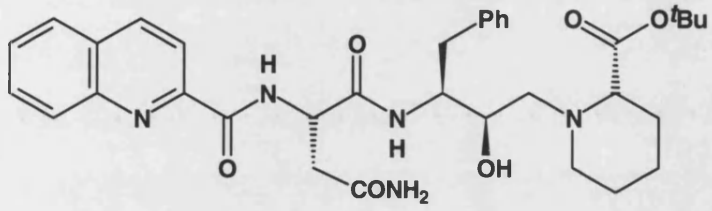

a) DIPEA, DMF, TFA-Pro-Ile-Val-$OCH_2$-Pam-Resin; b) $NaBH_4$, THF; AcOH; c) repeated cycles of deprotection/acylation; d) $Ac_2O$, DIPEA, DMF; e) HF, *p*-cresol, $0^oC$.

**Scheme 56**

Cieplak and Kollman[128] studied the free energy perturbation and molecular dynamics to predict the consequence of replacing each of the seven peptide bonds in the JG-365 *N*-methyl inhibitor. As yet, no definitive results have been published.

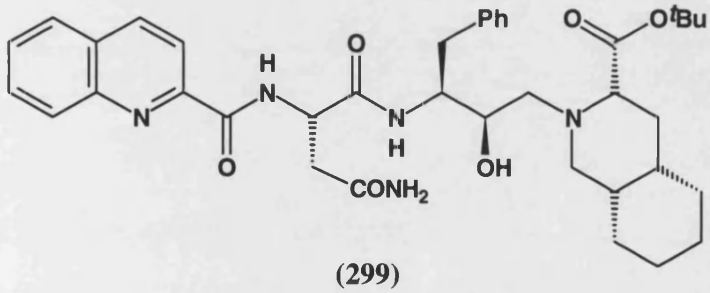
Kröhn *et al*[129] prepared numerous hydroxyethylamine peptidomimetics with intention of finding a potent HIV-1 protease inhibitor. The most potent are shown in **Figure 16**.

$IC_{50}$ (nM)




2

**(298)**

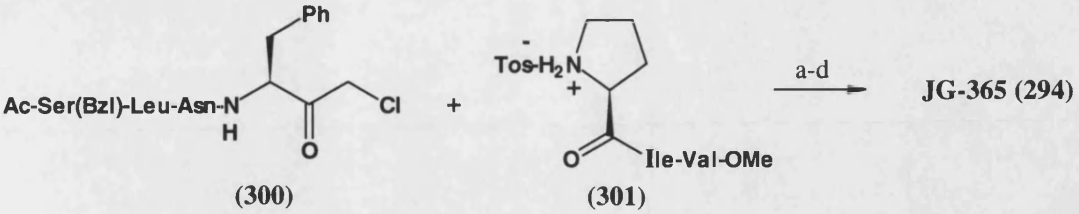



<0.4

**(299)**

**Figure 16**

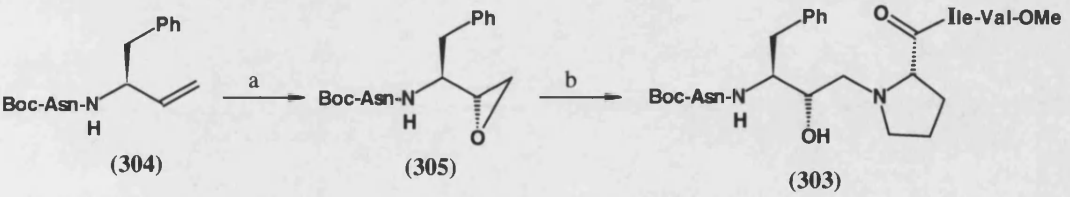
Rich *et al*[130] designed hydroxyethylamine dipeptidyl isosteres to mimic the transition-state for hydrolysis of Tyr-Pro, one of the partial substrate sequences cleaved by HIV protease. They also prepared the inhibitor JG-365 **(294)**. Their route involved coupling chloroketone **(300)** with Tos-Pro-Ile-Val-OMe **(301)**, reduction and then hydrogenation, giving the target inhibitor **(294)**, **Scheme 57**.




a. NaI, DMF; b. $NaHCO_3$; c. $NaBH_4$, MeOH; d. $Pd(OH)_2/C$, $H_2$, AcOH.

**Scheme 57**

Rich *et al*[131a] also prepared the (*S*)-configuration **(302)** of the inhibitor JG-365 **(294)** in a similar manner. They also prepared the inhibitor Boc-Asn-Phe-ψ [$CH(OH)CH_2N$]-Pro-Ile-Val-OMe **(303)** from the aminoalkene **(304)** in two steps, *via* epoxidation, separation and then epoxide ring-opening with HCl.Pro-Ile-Val-OMe, **Scheme 58**.

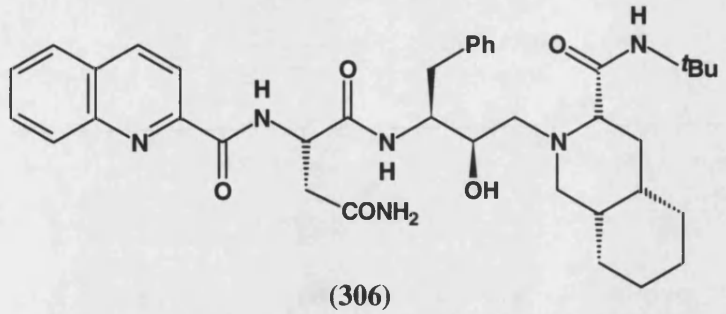



a) *m*-CPBA, DCM, 0°C, chromatographic separation; b) HCl.Pro-Ile-Val-OMe, $Et_3N$, MeOH, reflux.

**Scheme 58**

Rich *et al*[131b] also prepared the inhibitor **(299)** *via* an epoxide ring-opening reaction.

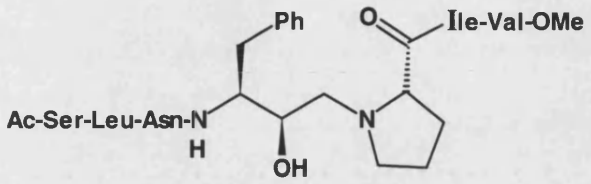
Many other research groups have utilised epoxide ring-opening reactions to prepare hydroxyethylamine isosters. These included work by Tucker *et al*[132] who prepared the HIV-1 proteinase inhibitors Ro 31-8959 **(306)**, JG-365(*S*) **(302)** and L-689,502 **(307)**, from the epoxide **(23)**, **Figure 17**.



(306)

$IC_{50}$ (nM)

**Ro 31-8959** >3000

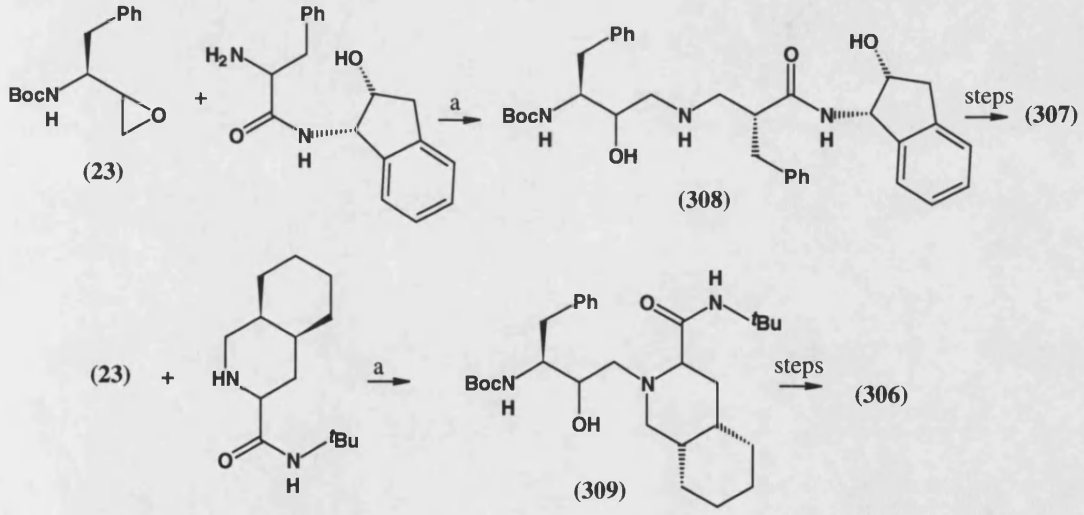


(302)

**JG-365 (*S*)** >3000

**Figure 17**

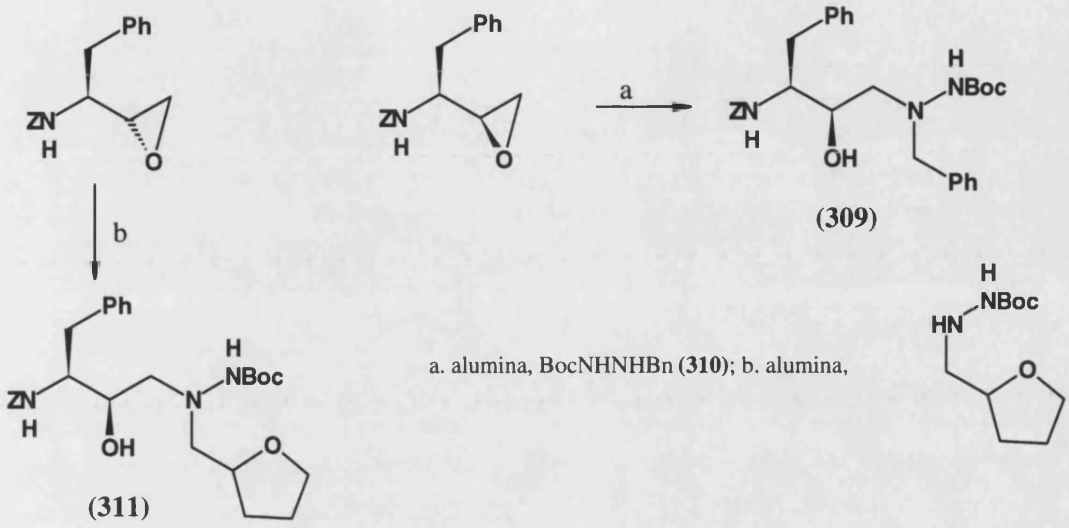
Epoxide **(23)** was ring-opened to give the amino alcohols **(308)** and **(309)**, these were converted to the appropriate inhibitor using standard peptide coupling procedures, **Scheme 59**.



a) W-200-N alumina, $Et_2O$, THF slurry, 72-96 hrs.

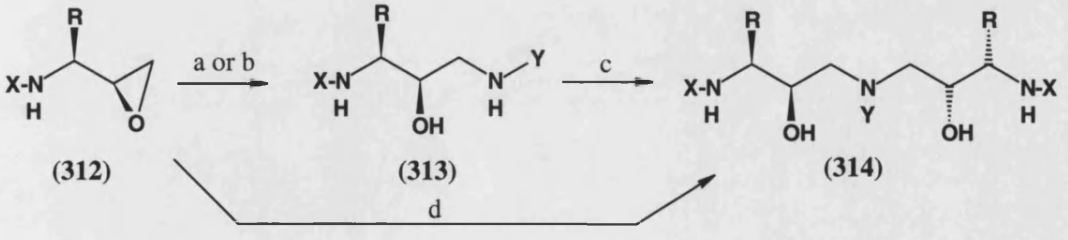
**Scheme 59**

Kempf *et al*[133] prepared the almost symmetric dipeptide **(309)** *via* the epoxide ring-opening by benzylhydrazine **(310)**, **Scheme 60**. This was shown to be a less potent inhibitor than hydrofuran analogue **(311)**.




**Scheme 60**

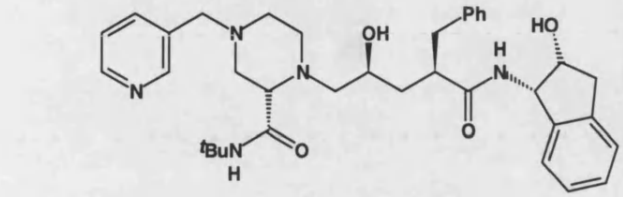
Gordon *et al*[134] developed a series of $C_2$ symmetrical inhibitors which were complementary to the $C_2$ symmetry of HIV protease homo dimer. The preparation of these inhibitors involved the epoxide **(312)** ring opening of selected amino epoxides, with ammonia and then reaction of this amino alcohol **(313)** with the same epoxide again to give the symmetrical amino alcohol **(314)**, **Scheme 61**.



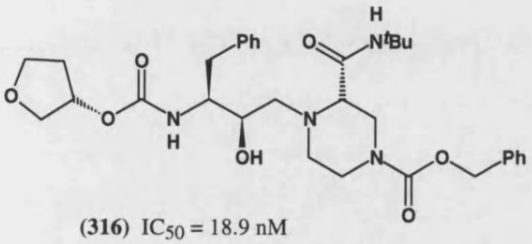

a. Y=H, $NH_3$, MeOH; b. Y=Bn, excess $BnNH_2$, DMF, 100°C; c. **(312)**, DMF, 100°C; d. 0.5 eq. $BnNH_2$, DMF, 100°C.

**Scheme 61**

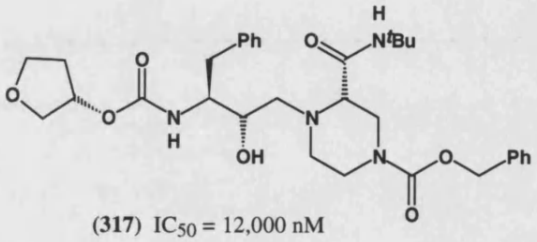
Kim and Huff *et al*[135] targeted HIV-1 protease for inhibition, and developed a new class of protease inhibitors of which L-735,524 **(315)** is a member. This placed the piperazine carboxamide unit at the C-terminal **(316)**, and found that the (*R*)-configuration was more tightly bound to the enzyme than the (*S*) isomer **(317)**, **Figure 18**. These analogues were prepared *via* epoxide ring-opening reactions.



**(315) L-735,524**



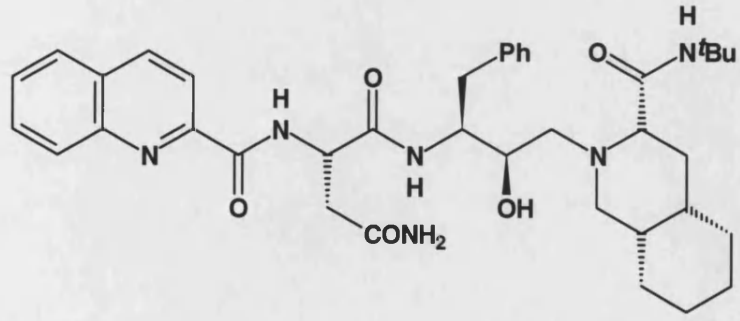
**(316)** $IC_{50}$ = 18.9 nM



**(317)** $IC_{50}$ = 12,000 nM

**Figure 18**

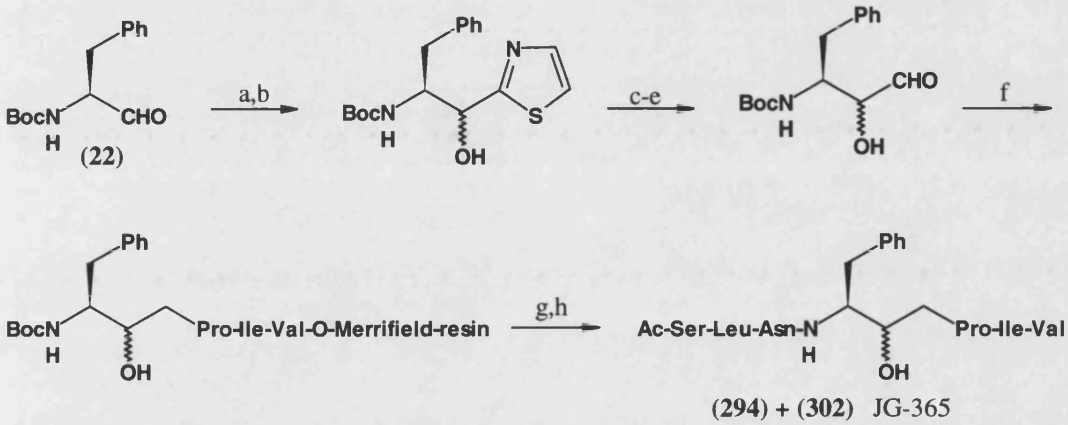
Parkes *et al*[136] prepared the potent HIV-1 inhibitor Ro 31-8959 **(318)**, which is currently in phase III of clinical trials. This hydroxyethylamine was also prepared *via* the ring-opening of *N*-protected aminoepoxide, **Figure 19**.



**(306) Ro 31-8959**

**Figure 19**

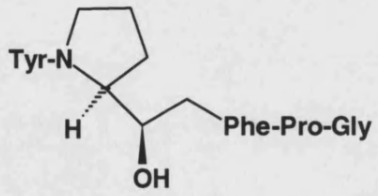
Tourwe *et al*[137] and Kim and Huff *et al*[138] prepared hydroxyethylamine inhibitors *via* reductive amination. Since Coy *et al*[21,34] developed the solid phase procedure for the preparation of reduced amide isostere (see **Section 1.4**), its use has increased enormously. Tourwe *et al*[139] adapted this procedure to prepare hydroxyethylamines with ease and high stereochemical control, **Scheme 62**.




a) 2-trimethylsilylthiazole, DCM, -30°C, 17 hrs; b) TBAF; c) MeI, $CH_3CN$, reflux; d) $NaBH_4$; e) $HgCl_2$, $CH_2CH{:}H_2O$ (4:1); f) $NaCNBH_3$, DMF, 1% AcOH, g) repeated cycles of deprotection and acylation; h) HF, anisole.

**Scheme 62**

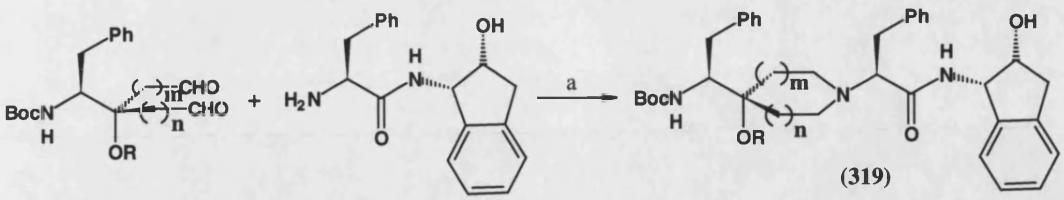
Tourwe *et al*[137] also used this procedure to prepare the inhibitor **(318)**, **Figure 20**.




**(318)**

**Figure 20**

Kim and Huff *et al*[138] prepared the secondary amine structure **(319)** similar to those of Tucker *et al*[132] using a reductive amination preparation, **Scheme 63**.




(319)

a) $NaCNBH_3$, AcOH, MeOH.

**Scheme 63**

However, all the secondary amine analogues were less potent than the corresponding hydroxyethylamine structures of Tucker *et al.*[132]

### 1.4.4 Other Peptide bond surrogates

Recently, Herranz *et al*[139] reported a new class of transition-state analogues. These contained the amide bond replacement ψ[CH(CN)NH], cyanomethyleneamino isostere. In this new peptide bond surrogate, they suggested that the cyano group keeps some *H*-bonding acceptor properties, while the new asymmetric centre could impart higher backbone rigidity than the reduced peptide bond. They prepared these analogues *via* Lewis acid catalysed reaction of *N*-protected α-amino aldehydes with a C-protected amino acid in the presence of TMSCN, **Scheme 64**.


**P=Z, Xaa = Phe, Yaa=Ala *R*:*S* 1:1**

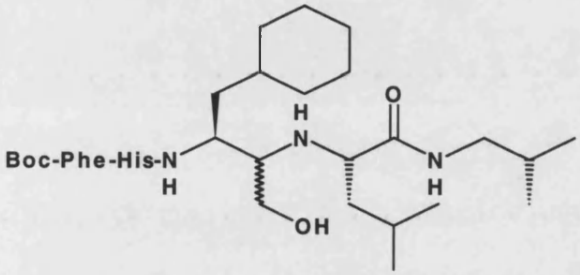
**P=Boc, Xaa=Phe Yaa=Pro *R*:*S* 9:1**

a) $Et_3N$, MeOH, $ZnCl_2$, -20°C, 1 hr, TMSCN, 0°C, 24 hrs.

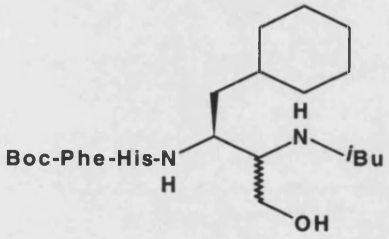
**Scheme 64**

Hangauer[140] prepared a new class of renin inhibitors, which incorporates the amide bond replacement ψ[$CH(CH_2OH)$], **Figure 21**. However, these were rather poor renin

inhibitors and their synthesis was not published. We hope to show that this new class may show some inhibitory activity with other enzymes, *e.g.* HIV-1, matrix metalloprotease and others. Our modelling studies, which will be discussed in **Chapter 3**, indicates that certain hydroxymethyl peptide analogues might be substrates for collagenase.




| | $IC_{50}$ (nM) |
|---|---|
| **(320)** | 4,400 |




| | |
|---|---|
| **(321)** | >20,000 |

**Figure 21**

## 1.5 Molecular Dynamics

Molecular dynamics procedure is a deterministic simulation process wherein the positions and the velocities of atoms in a molecule are integrated forward in time, using Newton's laws of motion. The initial velocities are randomly ascribed to atoms *via* a Maxwellian distribution consistent with the temperature at which the simulation is being performed. The movement of the atoms is then governed by the kinetic energy input into the system and the restoring forces that act on the molecule when its position from a minimum energy of the system can be determined. This term consists of strain energies; like bond length, bond angle deformations, torsional components *etc.* and interactions like van der Waals, non-bonding interactions and electrostatic terms. In this thesis, the Valence Force Field (VFF) is used to compute potential energy and the expression is given below. The potential energy of the compound studied is represented by the full valence force field (VFF).

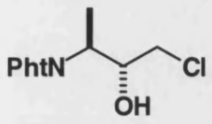
$$
\begin{aligned}
V \quad &= \Sigma\{D_b[1-e^{-\alpha(b-bo)}]^2-D_b\} \\
&+ \tfrac{1}{2}\Sigma H_\theta(\theta-\theta_o)^2 \\
&+ \tfrac{1}{2}\Sigma H\phi(1+s\cos n\phi) \\
&+ \tfrac{1}{2}\Sigma H_\chi\chi^2 \\
&+ \Sigma\Sigma F_{bb'}(b-b_o)(b'-b_{o'}) \\
&+ \Sigma\Sigma F_{\theta\theta}(\theta-\theta_o)(\theta'-\theta_{o'}) \\
&+ \Sigma\Sigma F_{b\theta}(b-b_o)(\theta-\theta_o) \\
&+ \Sigma F_{\phi\theta\theta'}\cos\phi(\theta-\theta_o)(\theta'-\theta_{o'}) \\
&+ \Sigma H_{\chi\chi'\chi\chi'} \\
&+ \Sigma\epsilon[(r^*/r)^{12}-(r^*/r)^6] + \Sigma q_i q_j/r
\end{aligned}
$$

The first four terms define the energy required to distort the internal from their ideal values ($b,\theta,\phi$ and $\chi$ are the bonds, valence angles, torsion angles and out of plane angles, respectively; $b_o$ and $\theta_o$ are the ideal bond lengths and valence angles and the H

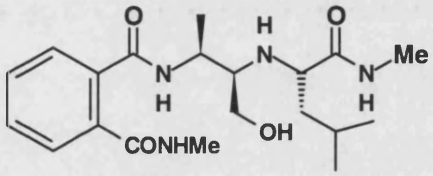
# NOMENCLATURE

## Stereochemical Notation and Compound Numbering

Throughout this thesis, the graphical representation of stereochemistry is in accord with the conventions proposed by Maehr[141] in 1985. Thus, solid and broken thick lines represent racemates and wedges denote absolute configuration where greater narrowing of the solid and broken wedges indicates increasing distance from the viewer.

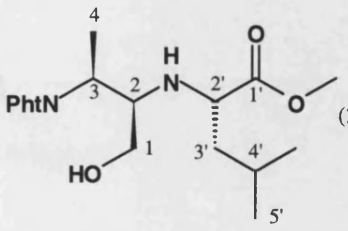



racemate




single enantiomer

The chemical nomenclature used throughout this thesis will be represented by a combination of that assigned in carbohydrate and in peptide nomenclature. The numbering system will follow that of the standard IUPAC convention. All amino acids mentioned are those of the natural stereochemical configuration, except were stated. Any text presented in italics represents that of similar work carried out by other co-workers in the organic chemistry department.




(2*R*,3*S*,2'*S*)-1-hydroxy-2-(*O*-methyl leucinyl)-3-phthaloylaminobutane

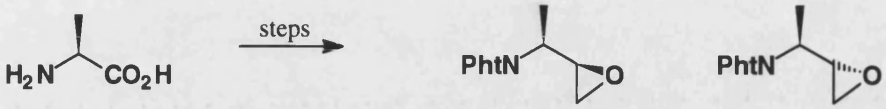
# CHAPTER TWO

# RESULTS AND DISCUSSION

## 2.1 Aims and objectives.

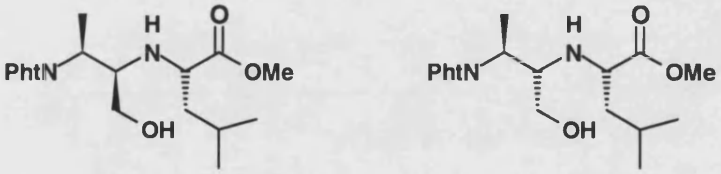
The objectives of this project were as follows:

a. The preparation of homochiral *N*-protected aminoepoxides **(322)** and **(323)** as key intermediates



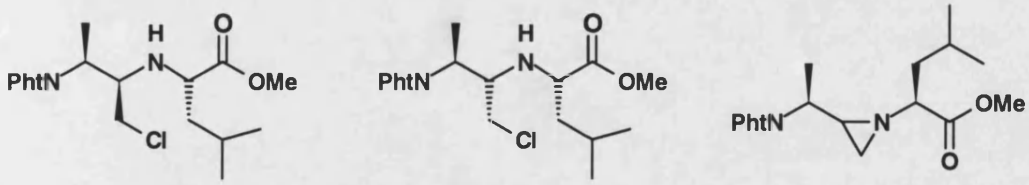

**(322)** **(323)**

b. The diastereoselective synthesis of the hydroxymethyl dipeptide mimetics **(324)** and **(325)**



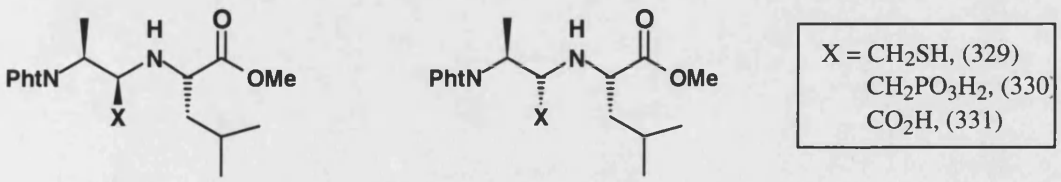

**(324)** **(325)**

c. The diastereoselective synthesis of the chloromethyl dipeptide mimetics **(326)** and **(327)** and the aziridinyl dipeptide mimetic **(328)**

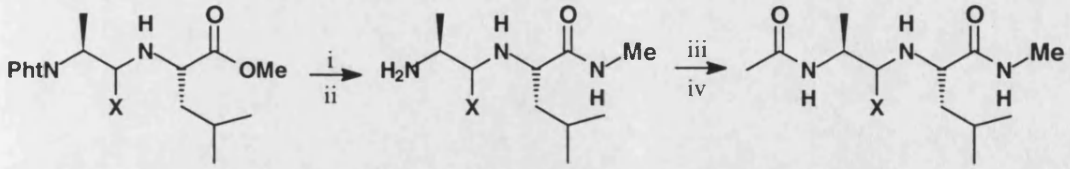



**(326)** **(327)** **(328)**

d. Diastereoselective synthesis of the mercaptomethyl, phosphonomethyl and carboxymethyl dipeptide mimetics **(329)**, **(330)** and**(331)**, and



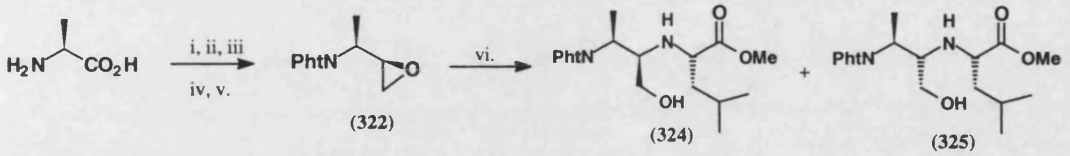
e. The conversion of the dipeptides to the corresponding pseudo-tetrapeptides **(332-336)**.



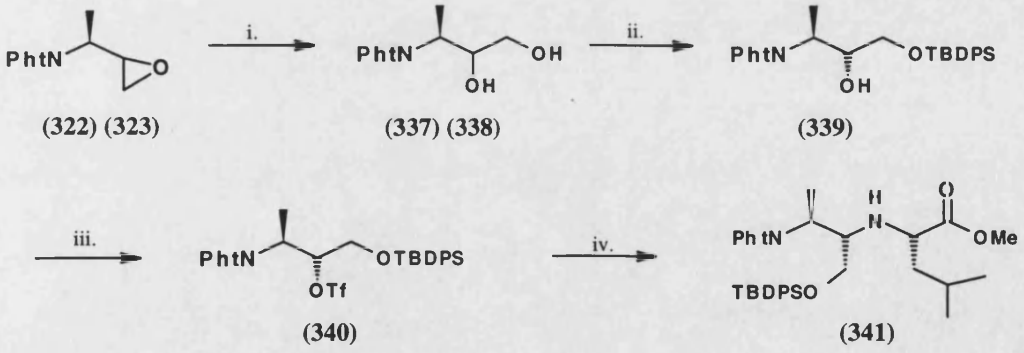
X = $CH_2OH$, **(332)** X = $CH_2PO_3H_2$, **(335)**
= $CH_2SH$, **(333)** X = $CH_2Cl$, **(336)**
= $CO_2H$, **(334)**

i. $MeNH_2$, MeOH; ii. $H_2NNH_2$, MeOH; iii. pyridine, $Ac_2O$; iv. deprotection.

The strategy would employ the ring opening of epoxide **(322)**, either to give the desired hydroxymethyl dipeptide **(324)** and **(325)** or the corresponding hydroxymethyl derivatives **(337-339)** which could then be activated, thus facilitating coupling with the required amino ester **(341)** (**Scheme 65**).



i. protection; ii. reduction; iii. oxidation; iv. olefination; v. epoxidation and separation; vi. acid catalysed ring opening with Leu-OMe.



i. acidic hydroxylation; ii. protection and separation; iii. activation with triflate; iv. coupling with Leu-OMe.

**Scheme 65**

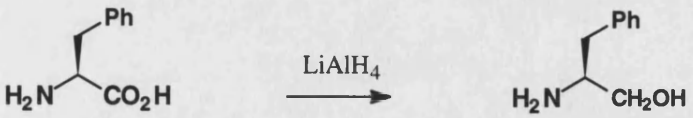
## 2.2 α-Amino aldehyde synthesis

### 2.2.1 Phthaloylamino protected route

In the area of peptide mimetics there has been an immense amount of research undertaken. Since the late 70's research groups have been preparing peptidomimetics from chiral amino acids, using a variety of protecting groups and various synthetic routes. A prodigious amount of groundwork has been covered by these groups and nowadays the preparation of peptide-mimetics has been made somewhat easier. However, there are still great problems associated with the optical activity of intermediate compounds, due to the labile nature of the acidic α-protons. Jurczak and Golebiowski[142] reviewed the synthesis of α-amino aldehydes in 1989. They showed that a variety of *N*-protected amino carboxylate derivatives had been successfully converted to the corresponding aldehydes in moderate to excellent yield. Other groups had reduced the α-amino acids directly to the α-amino alcohol and then oxidised them to the α-amino aldehydes. We had decided to use the later route to prepare our α-amino aldehydes. Although a vast amount of ground work had been achieved for the *N*-protected Boc[62,63,143] and Cbz[144] α-amino aldehydes, less attention had been paid to *N*-phthaloyl α-amino aldehydes.[145] We adopted this protecting group as it usually led to crystallinity, was stable to acid and base conditions, and potentially easily removed using either hydrazine,[146] $Na_2S$[147] or $NaBH_4$.[148] In the event we had to do extensive development work to overcome the problem of racemisation of the aldehyde and the following low yielding Wittig or related olefination step.

*In our laboratory starting from commercially available phenylalanine (342), reduction to phenylalaninol (343) by use of lithium aluminium hydride*[143h] *gave the corresponding alcohol at best in a 66% yield* (**figure 22**). Lithium aluminium hydride is pyrophoric and an extremely powerful reducing agent. On relatively large scale

reactions it could prove to be potentially hazardous. In an attempt to improve the yield and to avoid this hazard, a milder reducing agent was sought.



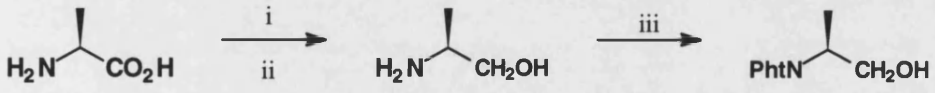

(342) (343)

**Figure 22**

Unlike powerful reducing agents such as lithium aluminium hydride, the mildness of borane is such that reductions of carboxylic acids can be undertaken in the presence of other functionality's *e.g.* ester, nitro, nitrile and keto groups. It was reported by Brown *et al*[149] that carboxylic acids are reduced rapidly to the corresponding alcohol in 100% conversion (80% by isolation) using borane-tetrahydrofuran complex.

*Preparation of alaninol* (**344**).

Borane-THF complex was employed to reduce alanine (**345**) to the corresponding alcohol (**344**). Three equivalents were used, one borane unit to reduce the acid and the other two to complex with the nitrogen. After a day at room temperature the reaction was quenched using sodium hydroxide and then stirred at room temperature for a day to ensure that the borane-nitrogen complex was fully cleaved (**Scheme 66**).



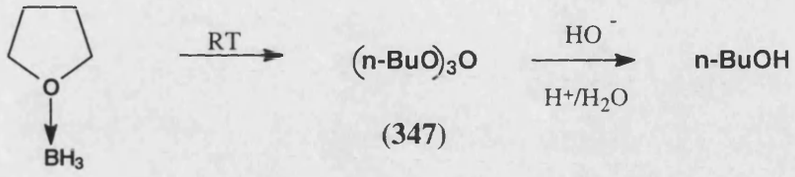

(345) (344) (346)

i. $BH_3$-THF, THF, 4½ hrs., RT; ii. 3M NaOH, 12 hrs., RT, 63%; iii. phthalic anhydride, 170°C, 2 hrs, 55%.

**Scheme 66**

Purification by distillation did not yield the expected alcohol (**344**) but rather butanol, which was identified by it s characteristic odour and by spectroscopic techniques. It was probably formed by a hydride transfer reaction at room temperature. The quality of the commercial borane proved to be irregular. The borane, which is complexed to the oxygen of THF, can supply a hydride ion which reductively ring opens THF. This

has been reported previously by Brown *et al,*[150] when the borane-THF was heated to 60°C for 64 hours giving a 64% conversion. This may happen slower at lower temperatures which ultimately results in the tributyloxy borane complex (**347**), which when reacted with water produces butanol (**Scheme 67**).

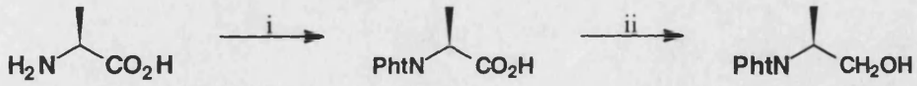



(347)

**Scheme 67**

When the reduction of alanine (**345**) wuth borane was repeated and the product purified by flash chromatography, the yield of alaninol (**344**) was surprisingly low. This was found to be due to its volatility. The isolated alcohol (**344**), was protected by the procedure outlined by Itoh *et al.*[151] The phthaloylamino alcohol (**346**) was formed in 55% yield, together with some phthalic derivatives which were difficult to separate by chromatography from the product due to similar retention factors. The overall yield for the first two steps was disappointingly low (34%). Protection of the unpurified alcohol, in a one pot reaction, gave the phthaloylamino alcohol (**346**) in an improved overall yield of 45%.

A different approach to the synthesis of the protected alcohol (**346**) was detailed by Becker *et al.*[145a] This involved protection first and then reduction to the alcohol (**346**) using borane-dimethylsulfide complex. (**Scheme 68**)




(345) (348) (346)

i. phthalic anhydride, 120°C, 7 hrs, 99%; ii. a. $BH_3$-DMS, THF, RT, 2 days, 63%; b. $BH_3$-THF, THF, RT, 2 days, 88%; c. $BH_3$-THF, THF, 40°C, 2 hrs., 64%.

**Scheme 68**

*Preparation of the N-phthaloyl-alanine* (**348**).

Protection required heating alanine and phthalic anhydride together for several hours. **Table 3** shows the effect of temperature on optical activity. Fusing the two together at a higher temperature than suggested (170°C) speeded protection up (2 hours) but resulted in racemisation. The best optical activity was achieved when the reaction was heated to 120°C as reported by Becker *et al.*[145a] The protection worked very well and the *N*-phthaloyl-alanine (**348**) was isolated in 99% yield by simply washing out the unreacted amino acid (**345**).

**Table 3**

| Temperature °C | Time (hrs) | Optical rotation (in EtOH) |
|---|---|---|
| 170 | 2 | -5.6°, *c* 0.753 |
| 140 | 6 | -9.0°, *c* 1.008 |
| 130 | 5 | -23.3°, *c* 1.218 |
| 120 | 7 | -24.7°, *c* 1.250 |

Bose, Greer and Price[152] in 1958 reported the synthesis of *N*-phthaloyl-alanine (**348**) using phthalic anhydride in toluene with triethylamine as a base. The conditions were slightly milder than those described by Becker *et al*,[145a] the advantage being that the reaction temperature only reached the boiling point of toluene. When this was attempted the optical activity was comparable to that given by Becker. From these results either method was satisfactory.

*Preparation of the of the N-phthaloyl-alaninol* (**346**)

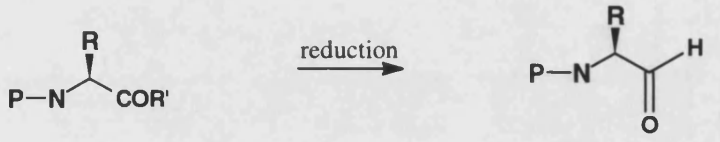
Reduction of *N*-phthaloyl-alanine (**348**) to the alcohol was achieved by using one equivalent of borane-tetrahydrofuran complex (a third of the amount that was used before). The alcohol (**346**) was produced in high yield (88%). Purification was vastly simplified as there were no phthalic by-products formed. Overall yield was now 88%.

Brown[153] reported in 1979 that if the borane reduction step was employed at elevated temperatures, the reaction would go to completion more rapidly. When this was tried at 40°C all the acid had been consumed, after 2 hours and the amino alcohol **(346)** was isolated in 64% yield.

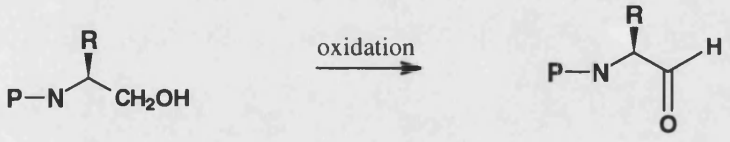
### 2.2.2 Oxidation of *N*-protected alaninol (346) and (349)

*Preparation of N-phthaloyl-alaninal* **(350)**

The preparation of *N*-phthaloyl-alaninal **(350)** has been achieved in three major ways; reduction of α-amino carboxylate derivatives,[62,63,142,143,144] oxidation of α-amino alcohols[62,63,142,143,144] and hydroformylation of α-amino alkenes.[145] Our efforts were concentrated on the oxidation of the alcohol **(346)** to the corresponding aldehyde **(350)**. (**Scheme 69**)

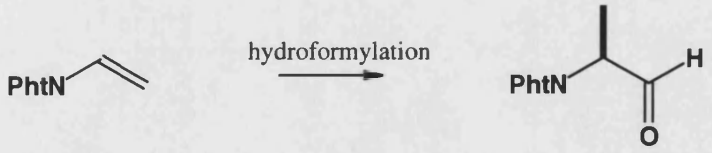



**(351)** **(352)**




**(353)** **(352)**

R = alkyl, R' = alkyl or leaving group and P = Boc, Cbz and PhFl




**(354)** **(350)**

**Scheme 69**

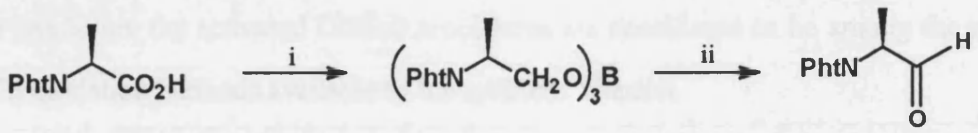
It has been reported by Stanfield *et al*[143d] that pyridinium dichromate (PDC)[154] is suspected of causing racemisation when *N*-protected amino alcohols are oxidised.

However, pyridinium chlorochromate (PCC)[155] has been reported by Fuchs *et al*[156] not to suffer from this problem as an oxidising agent. When this was applied to our system, we employed 5 equivalents of PCC, stirring at room temperature for 1 day. The amino aldehyde **(350)** was isolated after chromatography in good yield (on a small scale, 7mmol, 78% yield). On a larger scale the yield was considerably reduced (15-50% yield), **Table 4**.

To produce a large amount of the aldehyde **(350)**, the alcohol **(346)** was reacted in several portions. Each portion was individually oxidised, worked up, and then combined ready for olefination. Using the portioning technique was tedious and time consuming and the problem with low yield was still associated with the work up. Brown *et al*[153] suggested that reduction and oxidation in one pot *via* the trialkoxy borane **(355)**, could improve the yield of the desired aldehyde, (**Scheme 70**).

**Table 4**

| Alcohol (mmol) | support agent | equiv.s | (crude yield %) isolated % | time (hrs) | temp. °C |
|---|---|---|---|---|---|
| 7 | none | 5 | 78 | 24 | 21 |
| 25 | none | 5 | 50 | 24 | 21 |
| 94 | none | 5 | 15 | 24 | 21 |
| 15 | none | 2 | (70) | 5 | 40 |
| 20 | none | 2 | 46 | 2 | 40 |
| 97 | alumina | 2.8 | 90 | 2.5 | 40 |
| 29 | celite | 2 | (94) | 3 | 40 |
| 37 | celite | 2 | 67 | 4.5 | 40 |




**(348)** **(355)** **(350)**

i. $BH_3$-THF, THF, RT, 2 days; ii. PCC, DCM, reflux, 3 hrs, overall yield 30%.

**Scheme 70**

This was attempted on a relatively large scale. The yield was low, being only 30% again. The concept that adsorbed reagents on an inert inorganic support enhance reactivity of reagents has been employed by Cheng *et al.*[157] They used alumina as the support and adsorbed PCC onto its surface. This had the effect of enhancing the oxidation of the alcohols, and the use of alumina simplified the work up to a simple filtration. We utilised this property to oxidise alcohol **(346)** on a large scale, using two equivalents of PCC. The reaction was refluxed in DCM and was completed in two hours. After work up the aldehyde **(350)** was isolated in 90% yield (for a 0.035 molar scale reaction). T.l.c. of isolated samples showed marked streaking on the plate. Further investigation by t.l.c. (varying eluent) showed that in most cases the aldehyde was contaminated by unreacted alcohol and phthalic derivative(s) which run on either side of the aldehyde. Chromatographic purification gave the aldehyde **(350)** in high purity. Another PCC oxidation method used celite[158] as a support agent to prevent loss of material and simplify the work up. This worked very well when the reaction was refluxed, but again by-products formed and this made it difficult to purify the aldehyde. Studies of the optical activity of the PCC derived amino aldehyde **(350)** showed that all but those done at room temperature had been racemised to a certain degree. With respect to this problem and the difficulties encountered with purifying the product we turned our attention to an alternative oxidation method.

*Activated dimethyl sulfoxide oxidation reactions.*[159]

Owing to the uncomplicated procedure, simplicity of product isolation, high yield, mild conditions and subsequent absence of major side reactions including racemisation and

over oxidation, the activated DMSO procedures are considered to be among the most useful oxidation methods available to the synthetic chemist.

Parikh-Doering oxidation.[143h,160]

The Parikh-Doering method is probably the best documented activated DMSO oxidation procedure. This has the advantage that it is inexpensive and can be carried out at room temperature. When we attempted this we were hoping for complete conversion of the alcohol to the aldehyde **(350)** but unfortunately the reaction did not go to completion, with only 7% of the product (93% alcohol) being observed by n.m.r.

Swern oxidation[161]

The Swern oxidation proved to be the best method for the preparation of the desired aldehyde independent of molar scale. The yields were almost quantitative (92-98%), and after work-up the aldehyde **(350)** was pure enough to be used directly in the next step. The rate of addition of triethylamine was important in terms of optimising optical activity, as was the requirement for the complete removal of excess base during work up. Use of hindered base, *i.e.* diisopropylethylamine (DIPEA)[162] also helped to reduce the problem of racemisation during work up. In our hands, using this base, the aldehyde **(350)** was isolated enantiomerically pure $[\alpha]_{589}^{20}$ -38.6 in 72% yield.

TPAP oxidation[163]

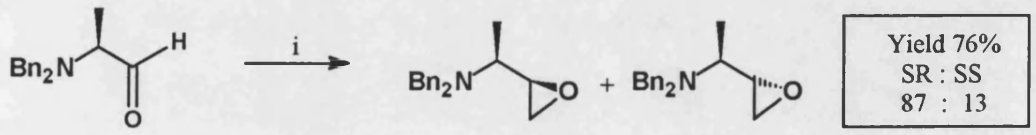
In 1987 Ley *et al*[163] introduced tetrapropylammonium perruthenate (TPAP) as a catalytic reagent for the oxidation of alcohols to aldehydes and ketones under very mild conditions with a simple work up that should not cause any racemisation. When this was attempted the reaction did not go to completion (only 35% converted), but the optically integrity was kept.

*The physical nature of N-phthaloyl-alaninal* **(350)**

The aldehyde **(350)** was found to be oxidised quite readily when exposed to the atmosphere and racemisation occurred readily at room temperature. After two months at room temperature in benzene the optical rotation had fallen to $[\alpha]_{589}^{24}$ -14.3° from $[\alpha]_{589}^{20}$ -38.6°. Under an inert atmosphere at reduced temperature decomposition still occurred but at a much slower rate. It could be stored at -17°C for several days without marked racemisation. Due to the unstable nature of the aldehyde **(350)**, it was always used immediately.

### 2.2.3 *N,N*-Dibenzyl protection procedure

Due to the low yielding preparation of *N*-phthaloylamino epoxide **(322)** and **(323)** *via* the Wittig olefination,[143h] a new protecting group was considered. Reetz *et al*[164] demonstrated that the *N,N*-dibenzylamino epoxides **(356)** and **(357)** can be formed in high yield from the corresponding aldehyde **(358)**, (**Scheme 71**), by using sulfonium ylides of the type $Me_2S{=}CH_2$.[64]




**(358)** **(356)** **(357)**

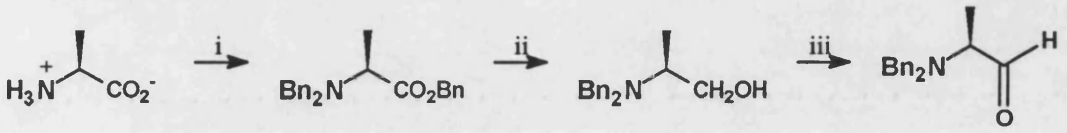
i. NaH, DMSO, 55°C, 1 hr., $Me_3S^+I^-$, -10°C, 30 mins.

**Scheme 71**

*Preparation of the dibenzyl protected Ala-OBn* **(359)** *and alaninol* **(349)**

**Scheme 71** shows the synthetic route employed for the preparation of the *N,N*-dibenzylamino epoxides **(356)** and **(357)**. The synthetic route used to make the amino aldehyde **(358)** followed that detailed by Reetz *et al.*[165] From commercially available alanine, protection with benzyl bromide gave the *N,N*-dibenzylamino benzyl ester **(359)**

in 97% yield. Previous methods used benzyl chloride[166] which gave the product in 85% yield. Reduction with $LiAlH_4$ gave the desired protected amino alcohol **(349)** in good yield (81%) and high enantiomeric purity. The benzyl alcohol was removed by distillation as the two alcohols had almost identical t.l.c. retention factors. Oxidation to the corresponding aldehyde **(358)** was achieved most conveniently by Swern oxidation. **(Scheme 72)**



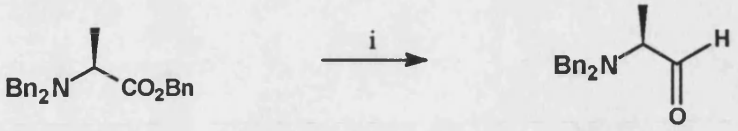

**(359)** **(349)** **(358)**

i. BnBr, NaOH, $K_2CO_3$, EtOH, $H_2O$, reflux, 97%; ii. LiAlH4, THF, reflux, 81%; iii. Swern, 89%.

**Scheme 72**

*Preparation of the dibenzyl protected alaninal*

Using PCC for the conversion of alcohol **(349)** to aldehyde **(358)**, resulted in low yield (9%). This was a result of a combination of benzylic oxidation (as a large amount of benzaldehyde was isolated) and complexation of the *N,N*-dibenzyl protecting group with PCC, thus trapping it in the residual oxidant. The alcohol **(349)** was also oxidised using Ley's perruthenate oxidising reagent TPAP.[163] This gave the aldehyde **(358)** in 72% yield after flash chromatography, with benzaldehyde again being isolated as a by-product of benzylic oxidation. Swern oxidation was by far the superior method, with oxidation cleanly giving the desired aldehyde **(358)** in good yield (89%). The overall yield from alanine was 70%, an improvement on the 44% achieved by Reetz *et al.*[167] Another possible way of producing the aldehyde **(358)** was to go straight from the ester **(359)** to the aldehyde **(358)** using DiBAL-H.[143a,143h,168] This was attempted on the benzyl ester **(359)** at -78°C in toluene. Unfortunately only 15% of the aldehyde **(358)** was isolated, **(Figure 23)**. This was believed to be because of the steric bulk of the benzyl group, and it was considered reducing the size of the ester to a methyl ester **(360)** may facilitate reduction to the aldehyde **(358)**.



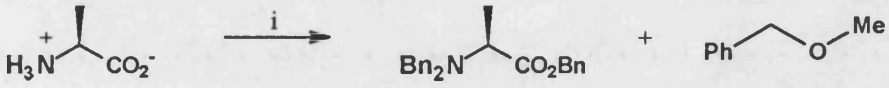

**(359)** **(358)**

i. DiBAL-H, -78°C, toluene, 30 mins., 15%.

**Figure 23**

*Preparation of the dibenzyl protected alanine methyl ester* **(360)**

It has been reported in a patent[169] that the methyl ester **(360)** could be generated by employing the benzylation in methanol. This was attempted, but only the benzyl ester **(359)** was isolated and benzylmethyl ether **(361)**. **(Figure 24)**

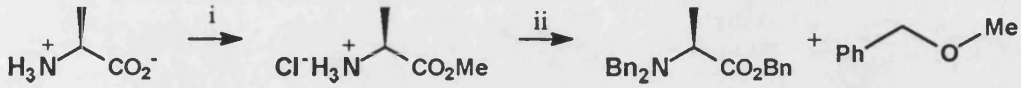



**(359)** **(361)**

i. BnBr, $K_2CO_3$, NaOH, MeOH, reflux.

**Figure 24**

These conditions were also used on the Ala-OMe.HCl **(362)** which was prepared quantitatively by treating alanine with methanolic HCl. Again only the corresponding dibenzyl ester **(359)** was isolated, along with the benzylmethyl ether **(361)**, **(Scheme 73)**. Attempts to convert the benzyl ester **(359)** to the corresponding methyl ester **(360)** in acidic methanol failed, with only the starting material being isolated.



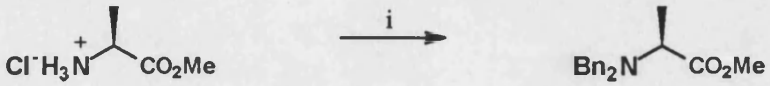

**(362)** **(359)** **(361)**

i. MeOH, HCl, 100%; ii. BnBr, NaOH, $K_2CO_3$, MeOH, $H_2O$, reflux.

**Scheme 73**

The methyl ester **(360)** was finally prepared in low yield by treating Ala-OMe.HCl **(362)** with potassium carbonate and benzyl bromide in acetone, using catalytic NaI to activate the alkylating agent. After two days there was no reaction, so three equivalents of triethylamine were added. This ended up giving only 11% of the desired

product after mild acidic work up. This yield was eventually improved to 32% by using triethylamine as the base in THF. (**Figure 25**)

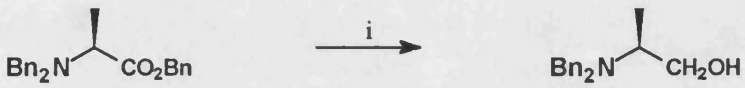



**(362)** **(360)**

i. a) BnBr, $K_2CO_3$, NaI, acetone, $Et_3N$, 3 days, 11%; or b) BnBr, $Et_3N$, THF, 1 day, 33%.

**Figure 25**

Reduction to the aldehyde using DiBAL-H at -78°C as detailed by Luly *et al*[143h] unfortunately went all the way to the alcohol (40%) and no aldehyde was isolated. (**Figure 26**)



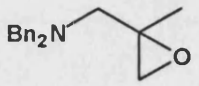

**(359)** **(349)**

i. DiBAL-H, -78°C, toluene, 30 mins., 40 %.

**Figure 26**

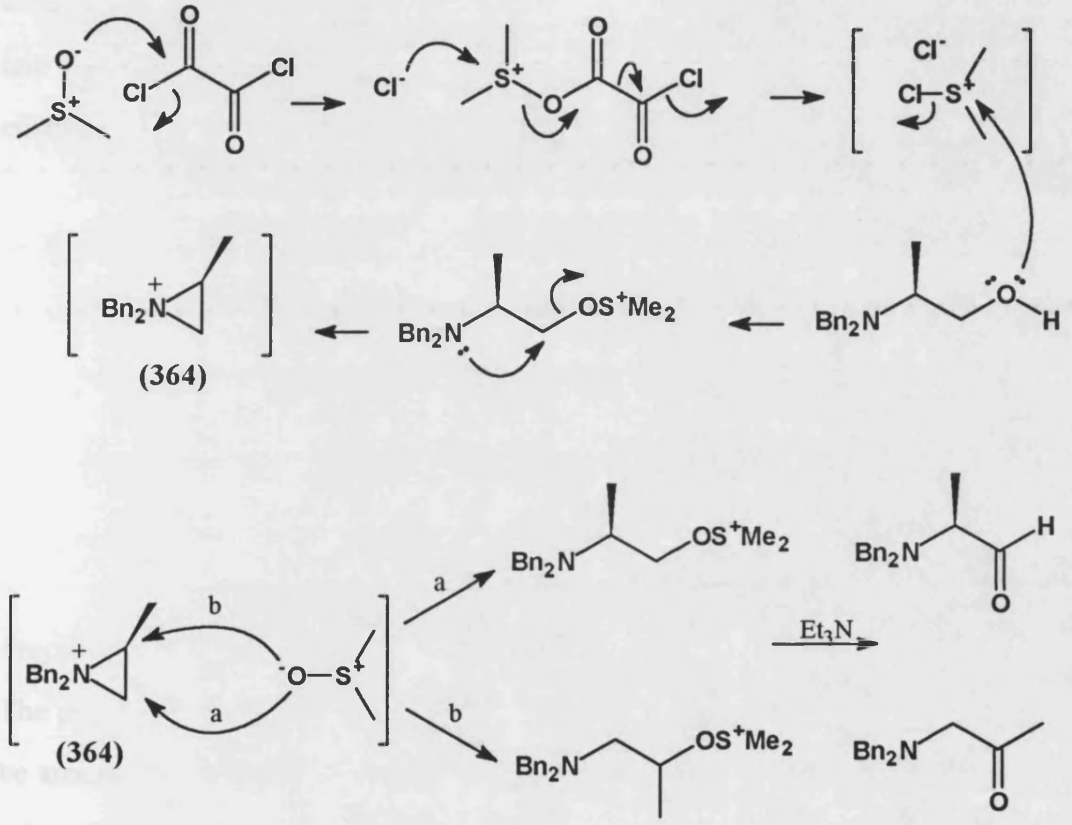
**Swern mediated isomerisation**

During the preparation of the *N,N*-dibenzylaminoepoxide **(356)** and **(357)** using the Swern oxidation, we discovered an unusual rearrangement. When we had prepared the aldehyde **(358)** using the standard Swern conditions, on one occasion, for no apparent reason (*i.e.* the conditions were not altered), we found that two compounds containing carbonyls (as visualised by DNP dip) were formed. We assumed incorrectly that one carbonyl was benzaldehyde, as there was always a small amount of residual benzyl alcohol left after purification of the $LiAlH_4$ reduction step. However, upon epoxidation it became clear from proton n.m.r. that there were two different isomeric epoxides **(356:357):(363)** in the ratio of 31:69 and that there was a singlet around the chemical shift expected for an α-methyl of alanine. Further analysis showed that this singlet was the signal for the rearranged epoxide **(363)** (see Section **2.4.2**). The isomerisation must have occurred




**(363)**

during the oxidation and one possible mechanism that may explain its formation is *via* an aziridinium salt **(364)**. The formation of such aziridinium salts has been reported by Rayner *et al*[170] (see Section **2.6.1**). The possible mechanism may involve the displacement of the sulfoxide group by the labile lone pair on the nitrogen **(Scheme 74)** to give the aziridinium salt **(364)**.



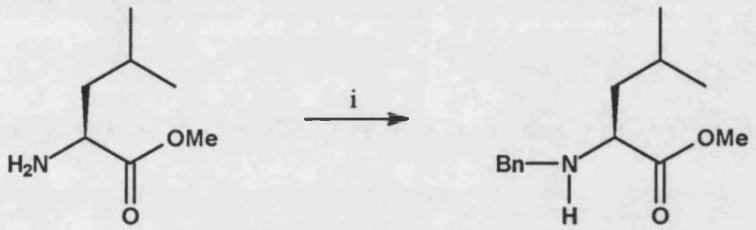

**Scheme 74**

The aziridinium salt **(364)** can then react with DMSO *via* two possible paths, either Path a, which regenerates the initial complex, or Path b which produces a new complex **(365)**. Upon addition of triethylamine, oxidative elimination occurred giving the two corresponding carbonyls **(358)** and **(366)**. We never isolated the methyl ketone **(366)**, and the only evidence for its existence is that it reacted with Brady's reagent, showing the presence of the carbonyl functionality and the fact that the *N,N*-dibenzyl methylepoxypropanes **(363)** were isolated together with the expected epoxides **(356)** and **(357)**. See Section **2.4.2** for the preparation of the epoxides.

### 2.2.4 Protection of Leu-OMe

*Preparation of the mono benzyl protected Leu-OMe* **(367)**

Leu-OMe was converted to the mono benzylamino methyl ester **(367)** in modest yield (57%), using similar conditions to that employed for the preparation of the dibenzylamino benzyl ester **(359)** as detailed by Velluz *et al.*[166] This protection used one equivalent of BnBr and $K_2CO_3$ as the base, refluxing for 2 days in THF and ethanol, (**Figure 27**).



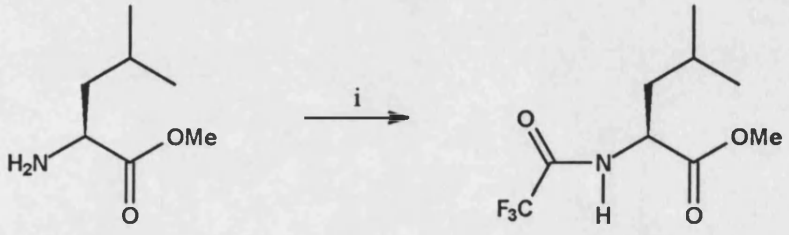

**(367)**

i. $K_2CO_3$, BnBr, THF, EtOH, reflux, 2 days, 57%.

**Figure 27**

*Preparation of N-trifluoroacetyl-Leu-OMe* **(368)**

The protected amino ester **(368)** was prepared because we believed that its $pK_a$ would be around 11, which is suitable for the Mitsunobu reaction. The protection involved using Leu-OMe.HCl with two equivalents of DIPEA and one equivalent of trifluoroacetic anhydride, **Figure 28**. The reaction went to completion in 16 hours, to give *N*-trifluoroacetyl-Leu-OMe **(368)** in quantitative yield. It was later discovered that its $pK_a$ was 10.4, experimentally determined at Glaxo Group Research, Ware.




**(368)**

i. DIPEA, trifluoroacetic anhydride, THF, 16 hrs, RT, 100%.

**Figure 28**

## 2.3 Olefination

Olefination of aldehydes and ketones can be achieved using a variety of stabilised carbanions. Probably the most widely used are phosphorus ylides due to their simplicity, convenience and efficiency. There are many non-phosphorus stabilised carbanions, such as trialkylsilyl groups[171] (Peterson), sulfoximides[172] (Johnson methylation), sulfones[173] (Julia olefination), boron[174] (Boron-Wittig), transition metals (Cr, [175] Zn, [176] Ti[177]), and other heteroatom stabilisers (chloromethyl-lithium,[178] tosyl-hydrazones[179] and reductive elimination of phenylthiomethylcarbinyl esters[180]). **Figure 29**.


| | |
|---|---|
| **X** = $Ph_3P$ | Wittig |
| $Me_3Si$ | Peterson olefination |
| S(O)(N=Me)Ph | Johnson methylation |
| $SO_2Ph$ | Julia olefination |
| $BR_3$ | Boron-Wittig |
| $[RCHCr_2^{III}]$ | |
| $CH_2I$-Zn-$Me_3Al$ | |

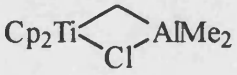



**Figure 29**

*Phosphorus stabilised ylides*

The essential principles of the Wittig[181-184] and related reactions[62,185,186] are the stabilisation of a carbanion by the phosphorus atom, reaction with a carbonyl compound, and subsequent elimination driven by the formation of the highly stable phosphorus-oxygen bond at the expense of the weaker phosphorus-carbon bond.

Phosphorus ylides fall into three main categories:- "Stabilised" ylides have strong conjugating substituents (*e.g.* $CO_2Me$, CN, or $SO_2Ph$) and usually favour the formation of the (*E*)-alkenes due to a late transition-state which resembles the

products; "semi-stabilised" ylides have mildly conjugating substituents (*e.g.* Ph or allyl) and often give no great preference for *E* or *Z* isomers; and "non stabilised" ylides that lack any stabilising functionalities favour (*Z*)-alkenes *via* a more reactant-like, early transition-state.

For our synthetic route we only required the addition of methylene and thus a non-stabilised ylide was used. It has been documented that *N*-protected amino aldehydes racemise under olefination, *i.e.* methyltriphenylphosphonium bromide methodology, [184] due to the labile α-proton. Luly *et al*[143h] demonstrated that *N*-phthaloyl amino aldehydes could be converted to the corresponding alkenes using methyltriphenyl-phosphonium bromide, *n*-BuLi in THF at -78°C to 20°C, without racemisation. In our hands, *N*-phthaloyl alaninal was converted to the corresponding alkene in low yield, 10-20%. This was not a satisfactory result, and another method was sought. Corey *et al*[182] showed that phosphorus ylides could be generated using NaH in DMSO at 0°C, producing the alkenes in good yield. Indeed, when this was applied to our system the yield was improved, but not substantially, to only 26%. It was also noted that the product had been totally racemised.

In all of the above Wittig reactions a large excess of methyltriphenylphosphonium bromide was used to convert the aldehyde to the corresponding alkene. In a review by Murphy and Brennan[187] it was reported that amides and imides **(369)** can be converted to olefins **(370)** (**Figure 30**).


**(369)** **(370)**

i. $Ph_3P{=}CH_2$, THF, 60°C, 60hrs.

**Figure 30**

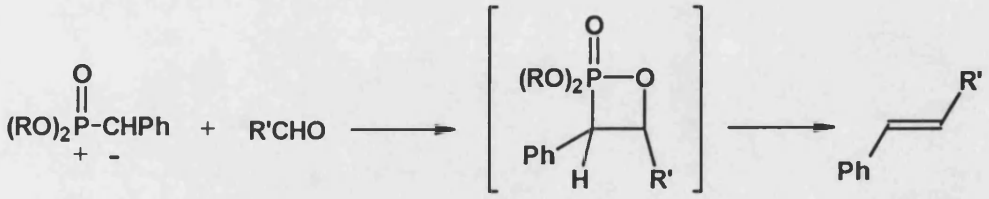
To overcome any possibility of this occurring, only one equivalent of base and methyltriphenylphosphonium bromide was used. **Table 5** shows the marked difference in yield for the Wittig reactions tried.

**Table 5**

| Metal base | Solvent | Equivalents | Temp. °C | Yield % |
|---|---|---|---|---|
| *n*-BuLi | THF | 2 | -78 | 13 |
| | THF | 1 | -78 | 38 |
| KO$^t$Bu | THF | 3 | 21 | 8 |
| | toluene | 1 | -20 | 65* |
| NaHMDS | THF | 5 | 0 | 6 |
| | toluene | 1 | 0 | 64* |

* Solvent effect is more important here

As the Wittig reaction was still low-yielding and the attempted epoxidation of the aldehyde using sulfur ylides (as detailed in Section **2.4.2**) was also low yielding, the olefination step was re-addressed. A modified Wittig, namely the Wadsworth-Emmons[62,185,186] which employs a dialkylbenzylphosphonate **(371)** stabilised carbanion, was reported to convert aldehydes to alkenes **(372)** in good yield, **(Figure 31)**.



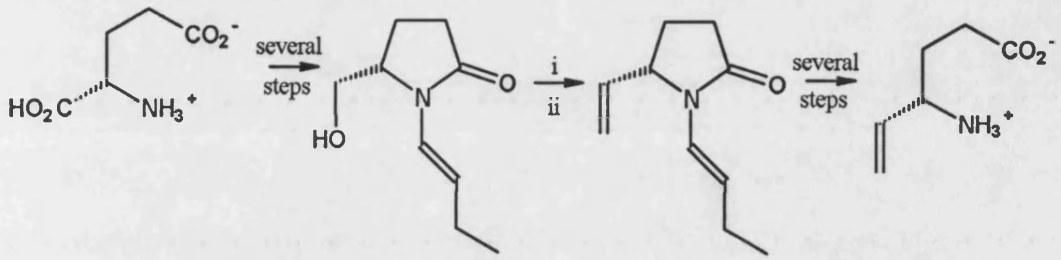

**(371)** **(372)**

**Figure 31**

The conditions involved using NaH or NaOR as the base in toluene or the corresponding alcohol respectively. In our hands using these conditions with

diethylmethylphosphonate, no alkene was isolated. This was undoubtedly due to the inability to form a sufficiently stable carbanion.

A more recent paper in 1992 by Smith *et al*[183] detailed the asymmetric synthesis of (4S)-aminohex-5-enoic acid **(373)**, using a similar preparative assembly to the one we had established in our programme. **(Scheme 75)**




**(374)** **(373)**

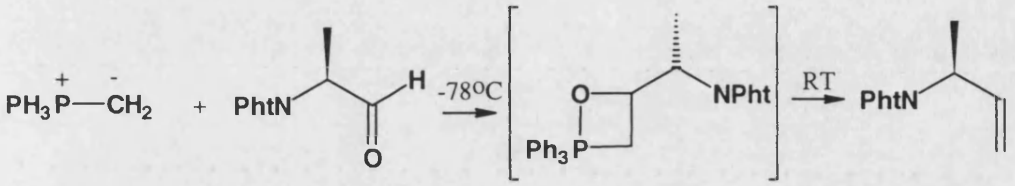
i. DMSO, DCC, pyridine, cat. TFA, 85%; ii. $Ph_3PCH_2Br$, KO$^t$Bu, THF, 77%.

**Scheme 75**

Smith also reported that *n*-BuLi in THF only gave 43% yield and NaH in DMSO at 80°C gave only 15% of **(374)**, but using KO$^t$Bu in THF at RT for 10 hours gave the best yield of **(374)** in 77% yield. When this was first attempted on our system, the alkene was obtained in extremely low yield (8%), when three equivalents of ylide were used in THF. When the solvent was changed to toluene and only one equivalent was used the yield was dramatically increased. With this very encouraging result we considered the effect other bases would have on the yield. It was reported by Bestman[188] in 1965 that it is important that the ylide formation should not involve lithium anions since the lithium salts react with the ylide to form complexes which strongly interfere with the reaction. This may explain why when using *n*-BuLi, the yield was always so low compared with KO$^t$Bu and NaHMDS. A much better method they suggested was to use NaHMDS[189] detailed by Wittig, Eggers and Duffner[190] in 1958.

Following these optimised conditions using NaHMDS in toluene at 0°C, the corresponding alkene was isolated in good yield (64-98%) see **Table 6**. Racemisation

did not occur when the ylide was added to the aldehyde at -78°C. It is very clear that the nature of the solvent had a great effect on the addition of the stabilised carbanion to the aldehyde. Polar solvents such as THF and DMSO must form a solvation cage which reduces the basicity of the carbanion, this in turn reduces the reactivity, disrupting the formation of the oxaphosphetane (**375**). **Scheme 76**.




**(350)** **(375)** **(376)**

**Scheme 76**

When toluene was used this problem was not encountered since toluene is less polar and does not interfere with the oxaphosphetane (**375**) formation. Eventual collapse of the intermediate at room temperature produced the alkene in almost quantitative yield.

**Table 6**

| Metal base | Solvent | temp[a] °C | yield % | $\alpha_D$ |
|---|---|---|---|---|
| *n*-BuLi | THF | -78 | 12 | 25.4 |
| | THF | -78 | 38 | 22.4 |
| | toluene | -78 | 56 | 25.4 |
| KO$^t$Bu | toluene | -20 | 65 | 12 |
| | THF | RT | 8 | N/A[b] |
| NaHMDS | THF | 0 | 6 | N/A |
| | toluene | RT | 98 | N/A |
| | toluene | 0 | 64 | 9.4 |
| | toluene | -78 | 92 | 23 |
| $NaCH_2S(O)Me$ | DMSO | 0 | 26 | N/A |

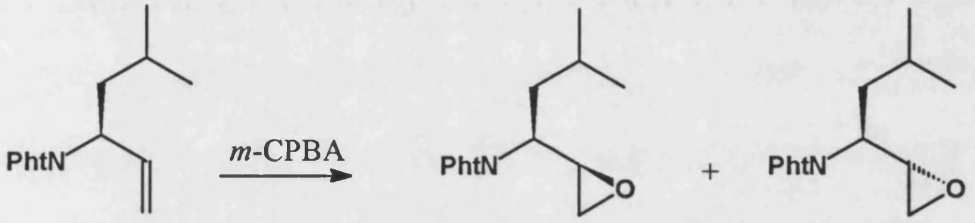
[a] temperature at which ylide was added to aldehyde

[b] racemic aldehyde used

## 2.4 Epoxidation

### 2.4.1 *m*-CPBA epoxidation

The most widely used epoxidising agent is *m*-CPBA, due to its high efficiency and its simplicity of use. Luly *et al*[143h] reported, in 1987, the use of *m*-CPBA in the synthesis of chiral *N*-protected amino epoxides. They reported that the epoxidation of (3*S*)-5-methyl-3-(phthaloylamino)hex-1-ene **(377)** provided predominantly the *threo* (*syn* addition) stereochemistry (**378:379**, 4:1, *threo*:*erythro*), **Figure 32**.

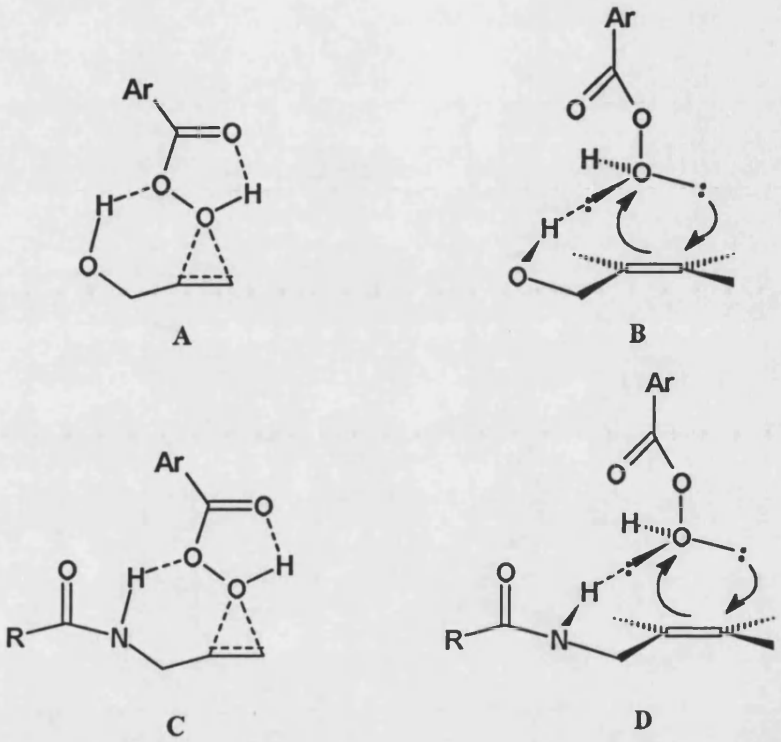



**(377)** **(378)** **(379)**

**Figure 32**

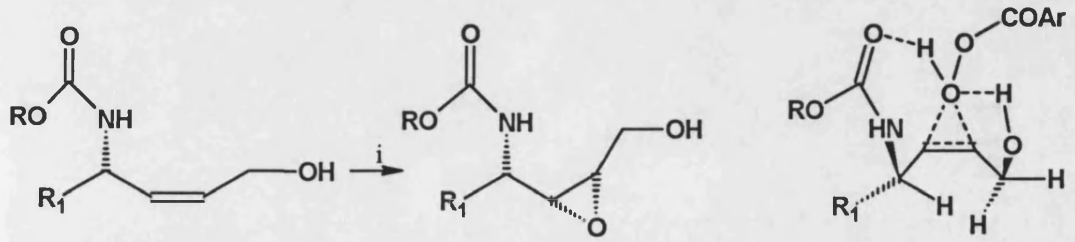
The stereochemical outcome has been discussed in a recent review by Evans *et al.*[191] They suggested that epoxidation could go *via* intramolecular delivery of the peracid (**Scheme 78**). Albeck and Persky[192] have also studied the epoxidation of *N*-protected aminoalkenes. Their argument for the high degree of stereoselectivity was also attributed to the directing effect of the amino group. They studied the epoxidation of terminal aminoalkenes and looked at the directing effects of allylic alcohols, amides and carbamates. This effect of reagent approach control was originally argued by Henbest in 1957, to be due to the hydrogen bonding between the hydroxyl proton of one of the oxygens of the peracid (A in **Scheme 77**). Sharpless later suggested a modified mechanism (B in **Scheme 77**), in which, on the basis of a detailed analysis of stereoelectronic effects, the hydrogen bond acceptor is the oxidising oxygen of the peracid. Roush *et al* suggested the steering effect came from the amido proton and

one of the oxygens of the peracid (C in **Scheme 77**). Finally, Kocovosky and Stary postulated a modified Roush's mechanism following Sharpless's acceptor model (D in **Scheme 77**).




**Scheme 77**

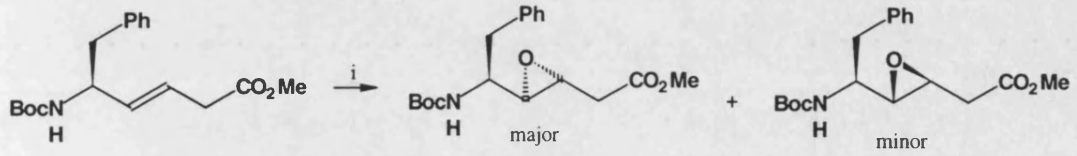
There are many other reports in the literature which have discussed the stereochemical epoxidation of *N*-protected aminoalkenes, Kogen and Nishi[193] followed the work of Kishi, who used *m*-CPBA to epoxidise *cis*-4-amino allylic alcohol **(380)**, where the carbonyl of the amino protecting group formed a hydrogen bond with the peracid **(381)**.




**(380)** R=Bn, **(383)** R=$^{t}$BuO **(382)** R= Bn, **(384)** R= $^{t}$Bu0 **(381)**

**Figure 33**

Kogen and Nishi showed that the epoxidation of *cis*-4-*N*-benzyloxycarbonyl-α-aminoalkene **(380)** gave exclusively the *syn* epoxide **(382)** (**Figure 33**). Ohfune and Hori[194] showed this was also true for *N*-Boc protected aminoalkenes **(383)** (**Figure 33**). Luthman[195] showed that the epoxidation of aminoallylic esters were highly selective due to the contributing co-ordination between the carbamate protecting group and the peracid. There was also a weak co-ordination effect between the allylic ester and the peracid (**Figure 34**).

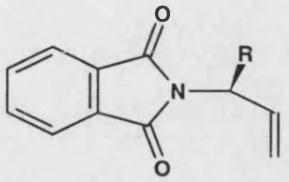



i. *m*-CPBA, (19:1).

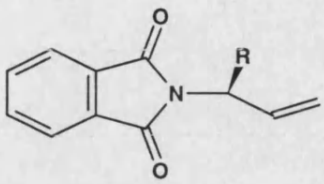
**Figure 34**

Assuming that the angle between the nitrogen, *alpha* carbon and the alkene is 120°, then the conformation of the amino alkene is dictated by steric hindrance effects of the R group. The amino alkene may adopt two possible conformations for intramolecular delivery of the peracid. These are the unfavourable eclipsed conformer, where the angle is -120°, and the less sterically hindered conformer, where the alkene is away from the R group with the angle at +120°. When the R=Bn, the less sterically hindered conformer may predominate, thus the peracid can hydrogen bond to the carbonyl of the imide and then approach from the top face With these aspects in mind the selectivity should be reduced when used with R=Me and indeed in practice the selectivity was low with *syn* addition still predominant 64:36, *syn:anti*. An example of a similar epoxidation is shown in **Scheme 78**. In this example the argument is much clearer as there is a conformational lock; $A^{1,3}$ strain is relieved by the methyl adopting an eclipsed conformation with the proton at the chiral centre. Here the peracid hydrogen bonds with the amide proton and addition is again directed *via syn* addition, the selectivity here is very high (>19:1 *syn:anti*).

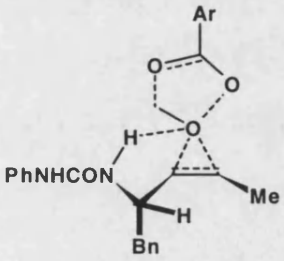
Rich and Romeo[196] have recently discussed the effect of diastereomeric enrichment during the epoxidation of *N*-protected aminoalkenes (see Section **2.5.4**). They postulated that the minor isomer decomposes faster to give an oxazolidine **(385)** and it is this process that is responsible for further diastereomeric enrichment.

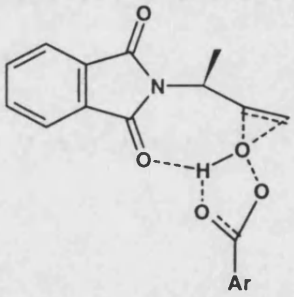


favoured if R is large



eclipsed, unfavoured





N-C-C=C $\Phi$=120°

Transition structure for the directed epoxidation of acyclic systems

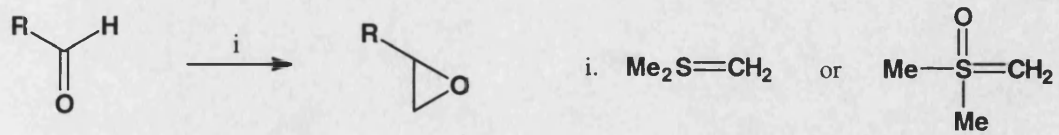
**Scheme 78**

In our hands, epoxidation using *m*-CPBA went quantitatively in the ratio 64:36 with isomer (2*R*, 3*S*) **(322)** as the major diastereoisomer.

### 2.4.2. Epoxidation using sulfur ylides

*Preparation of 3-phthaloylaminoepoxybutane* **(322)** *and* **(323)**

Corey *et al*[64] converted aldehydes directly to the corresponding epoxides using the sulfur ylides, dimethylsulfonium methylide **(386)** or dimethylsulfoxonium methylide **(387)** in good yield. (**Figure 35**)



i. $Me_2S{=}CH_2$ or $Me{-}S(=O){=}CH_2$ (Me)

**(386)** **(387)**

**Figure 35**

The sulfur ylide (**386**) can be generated from trimethylsulfonium salts by treatment with a strong base *e.g.* sodium hydride, *n*-BuLi or methylsulfinyl carbanion. The ylide is both aerobically and thermally unstable (rapid decomposition at temperatures above 10°C).[64,197] However, below -10°C the ylide is stable and can be utilised as a methylene equivalent for the conversion of aldehydes to the corresponding epoxides. Dimethylsulfoxonium methylide (**387**) is more stable at room temperature, and can be kept for several months at 0°C, under inert atmosphere without appreciable decomposition. This type of ylide can be generated in the analogous manner to that of the ylide (**386**). Its stability reduces its reactivity somewhat and it has been reported that some keto epoxidations do not occur.[64] Both ylides were used to generate the epoxides. **Table 7** details the conditions used and the yield and ratio of the epoxides isolated.

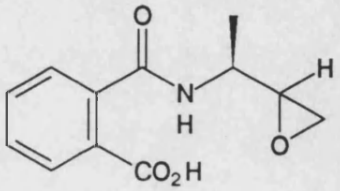
**Table 7**

| Ylide | Solvent | Yield % | Time (mins) | Ratio (**322**):(**323**) |
|---|---|---|---|---|
| Sulfonium (**386**) | THF | 2 | 30 | 68:32 |
| (**386**) | DMSO | * | 30 | - |
| (**386**) | THF | 10 | 30 | 55:45 |
| Sulfoxonium (**387**) | THF | 27 | 60 | 55:45 |
| (**387**) | THF | 18 | 120 | 55:45 |
| (**387**) | DMSO | 21 | 60 | - |

* many spots by t.l.c. negligible yield

The experimental procedure given by Corey for the epoxidation of benzaldehyde was followed. At extended periods of heating [55°C in the case of the sulfoxonium ylide (**387**)] yields were reduced. The best results achieved were for those epoxidations using the sulfoxonium ylide and a reaction time of one hour. Careful chromatography could separate the two isomers, thus they were fully characterised.

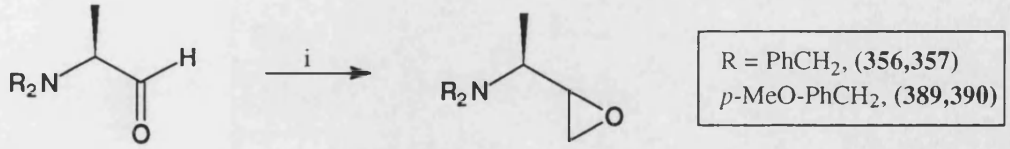
*Via* Corey's method for epoxidation of aldehydes, 27%. It was noted from the literature that epoxidation of aldehydes using sulfur ylides generated by metal hydroxides in two-phase systems, gave very respectable yields.[198,199] It was considered that these conditions may result in the phthaloylamino group being ring opened by the strongly basic conditions. These conditions are reported not to result in hydrolysis of the sulfonium salt or cause a Cannizzaro type redox of the starting aldehyde. A model reaction was tried with the phase transfer catalyst *n*-butylammonium iodide, used to assist the transfer of reagents between the two phases. The reaction was carried out at 50°C for 48 hours. After 2 days, the starting material had reacted, but only a small amount of the desired epoxide had been formed. T.l.c. showed some polar compounds and it was apparent that these were phthalic acid derivatives such as (**388**) formed by base ring opening of the protecting group. The reaction was abandoned without purification of the products.



(**388**)

*Preparation of the N,N-dibenzylamino epoxides* (**356**) *and* (**357**)

When the two phase epoxidation conditions were applied to the *N,N*-dibenzyl protected alaninal (**358**), there was no interaction of the base with the protecting group and subsequently the epoxides (**356**) and (**357**) were produced, in poor yield (12%).



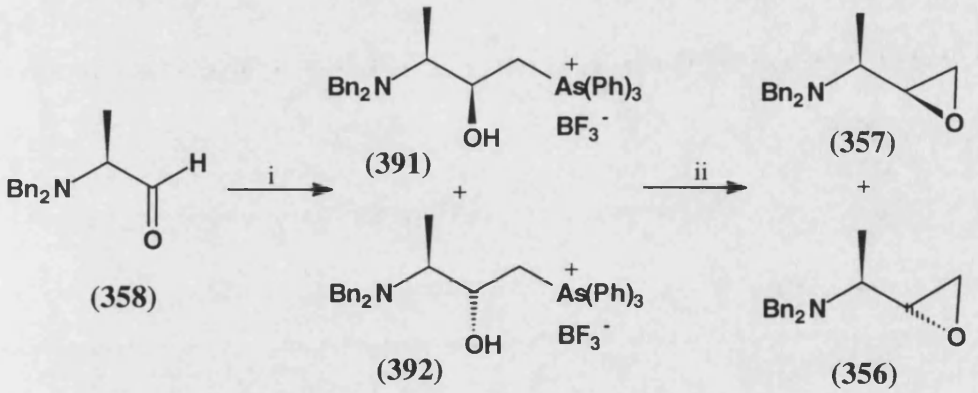

i. NaH, DMSO, $Me_3SI$, THF, -10°C.

**Figure 36**

Reetz *et al*[164] reported in 1989 that double protected amino aldehydes could be stereoselectively converted to the corresponding epoxides using sulfonium ylides of the type $Me_2S{=}CH_2$. The larger the protecting group the greater was the diastereomeric

excesses. For example, the benzyl protecting groups gave **(356:357)**, 87:13 whereas the 4-methoxybenzyl gave **(389:390)**, 91:9 (**Figure 36**).

The major diastereoisomer turned out to be the *erythreo* (or *anti*) adduct. The stereochemistry was assigned by reacting the crude epoxide mixtures with dimethyl copper (II) lithium, giving the corresponding amino alcohols. It was also reported that using arsonium ylides the diastereomeric excess was increased (**Table 8**), with these ylides it was not the epoxides that were isolated but rather the ylide adducts **(391)** and **(392)**, which were then converted to the corresponding epoxides, using NaH (**Scheme 79**).




i. $KN[Si(CH_3)_3]_2$, 10% citric acid, ii. NaH, THF, 45°C, 3hr.

**Scheme 79**

**Table 8**

| Epoxidation of double protected alanine | | |
|---|---|---|
| protecting group | yield of epoxide % | *threo:erythreo* |
| $PhCH_2$ | 73 | 87:13 |
| 4-MeOPh$CH_2$ | 50 | 91:9 |

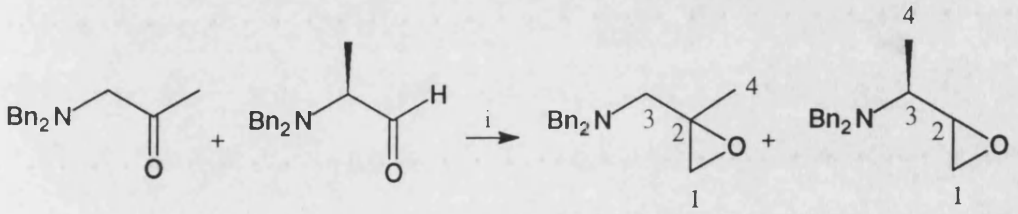
With this promising report, the dibenzyl amino aldehyde **(358)** was reacted with the sulfonium ylide $Me_2S{=}CH_2$ at -10°C. The corresponding epoxides were isolated as an

inseparable mixture in good yield (**356:357**), (13:87) (76%, 2*S*,3*R* as the major isomer as detailed by Reetz *et al*[164]).

*Preparation of the (2S,3S)- and (2R,3S)-dibenzylamino-2-methylepoxypropane* (**363**)

The Swern oxidation did not always give exclusively the corresponding aldehyde (**358**). On one occasion (as detailed in Section **2.2.3**) a rearrangement occurred giving the expected aldehyde (**358**) and the rearranged product, the methyl ketone (**366**).

**Figure 37**




(**366**) (**358**) (**363**) (**356**) , (**357**)

i. NAH, DMSO, 55°C, 45mins, MeSI, -10C, 5mins; then -10°C, 30mins, RT 1 hour.

**Figure 37**

These two products were successfully converted to the corresponding epoxides in good yield (83%) using the conditions employed by Reetz.[164] **Table 9** shows the comparison between the proton n.m.r.'s of the two epoxides prepared.

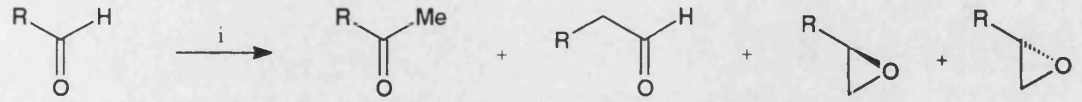
**Table 9**

| | chemical shift (ppm) | |
|---|---|---|
| Proton | Epoxides (**356,357**) | Epoxide (**363**) |
| 1 | 2.41 and 2.66 | 2.62 |
| 2 | 3.08 | - |
| 3 | 2.79 | 2.36 and 2.62 |
| 4 | 1.03 | 1.39 |

The proton n.m.r. spectra for the epoxide (**363**) showed that the methyl appeared as a singlet at 1.39 ppm, compared to the doublet at 1.03 ppm for the expected epoxide (**356,357**), that there was no H-2 signal, and that there was a quaternary carbon present in the carbon-13 spectra at 56.5 ppm. The proton n.m.r. also showed that the $CH_2N$ and $CH_2O$ protons were ABX systems, thus confirming the structure of the epoxide (**363**).

*Preparation of the epoxides using diazomethane*[200]

The insertion of methylene groups into aldehydes and ketones to give the corresponding epoxides and other carbonyl compounds have been well documented. In 1954 Gutse,[200] reviewed this area and showed that many aldehydes had been successfully converted to the corresponding epoxides in good yield. This carbene insertion reaction was attempted on aldehyde (**350**). Three possible products were expected to be seen, end insertion giving ketone (**393**), alkane insertion to give aldehyde (**394**) and carbonyl insertion to give the desired epoxides (**322**) and (**323**) (**Figure 38**). When this reaction was attempted, we did not isolate the aldehyde (**394**), but we did isolate the desired epoxides (**322:323**), (37:63) in low yield (7%) and ketone (**393**) as the major product (40%).




(**350**) (**393**) (**394**) (**322**) (**323**)

i. Diazomethane, R = PhtNCH(Me)-

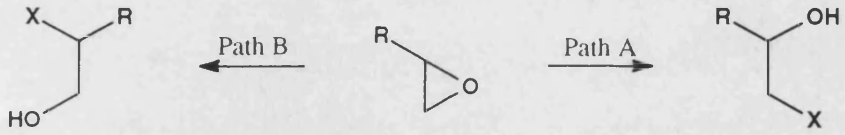
**Figure 38**

## 2.5 Epoxide ring opening

### 2.5.1 Addition of amino esters to *N*-protected oxiranes

*Preparation of the hydroxymethyldipeptide* (**324**) *and* (**325**) *via ring-opening of (2R,3S)- and (2S,3S)-phthaloylamino epoxybutane* (**322**) *and* (**323**).

Most attempts at ring opening of epoxides have involved the nucleophilic addition of amines to the least substituted carbon of an unsymmetrical epoxide, Path A, **Figure 39**. This requires heating the epoxide in an aprotic solvent, with excess amine. Many groups have established the synthesis of complex peptide mimetics utilising this effective synthetic method.[63,114,124e,131,133,136,201-203]



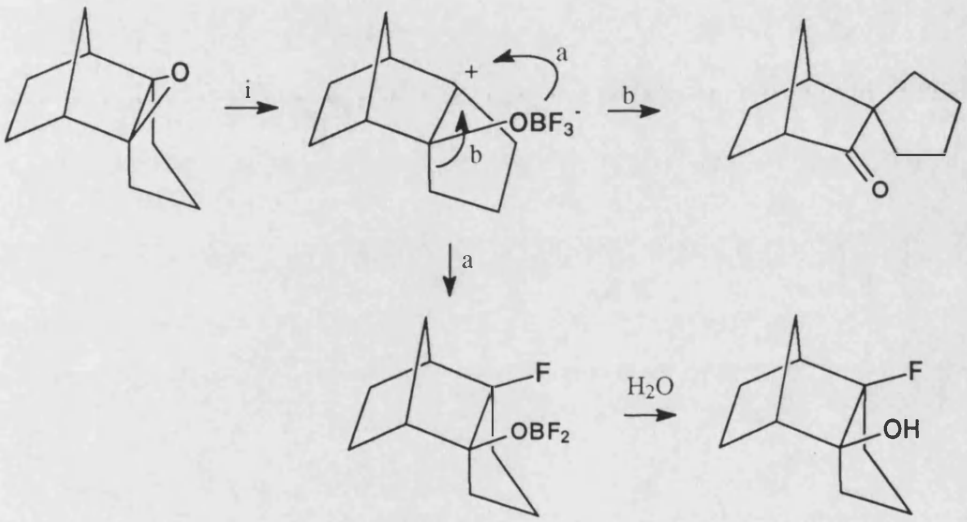

X = Halogen, nitrogen, oxygen or sulfur nucleophile

R = alkyl, and phenyl or allyl

**Figure 39**

Although this direct method is satisfactory in many cases, there are a number of limitations to the ring opening of epoxides with first generation amines ($R_2NH$ or $RNH_2$). These include; **i.** the low nucleophilicity of these amines require reactions to be carried out at elevated temperatures; **ii.** the regioselectivity is not always controllable; and **iii.** the reactivity of sterically bulky amines is low. To overcome these problems several metal amines have been developed. These are classed as second generation amines ($R_2NM$). There are many examples in the literature detailing the use of such second generation amine metals, *e.g.* Li, Mg, and Pb;[204] Al,[205] Cu,[206] Yb;[207] Ti and Sn.[208] All provide Path A products (**Figure 39**), as do $Al_2O_3$ mediated amination,[209] $Ti(OR)_4$-mediated ring openings of 2,3-epoxy alcohols and their

derivatives[210,211] and intramolecular cyclisations of epoxy amines and amides.[170,212] However, additions at the most substituted site, *via* Path B, have not been well established. These additions require the formation of a carbocation. There have been many reports in the literature where Lewis acids that consist of metal halides, insert a halide at the most substituted carbon of unsymmetrical epoxides *i.e. via* Path B. Such examples include $FeCl_3$,[213] $TiCl_4$ with DBU,[214] $BF_3.OEt_2$.[215] Other examples of this type of addition include the use of organotin amine compounds *e.g.* $Sn(Me)_3NEt_2$[216] and TMSCN which give a mix of Path A and B additions depending on the Lewis acid used.[217]

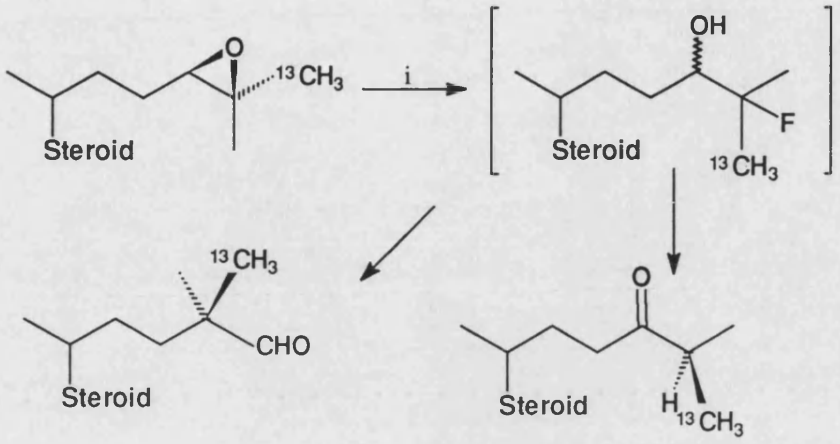



i. $BF_3.OEt_2$, DCM.

**Scheme 80**

It is interesting to note that Takaishi *et al*[215] reported that fluorine had been added to a tricyclic epoxide *via* transfer within a dipolar fluoroborate (**Scheme 80**). Other examples of fluoride additions using $BF_3.OEt_2$ have been reported, but all these examples are on carbocyclic systems. These results must be interpreted taking account of the factors inherent in cyclic systems, *i.e.* ring strain and axial-equatorial preference. With acyclic systems these problems are not apparent and thus this Lewis acid could be used to furnish amino alcohols. Fujimoto *et al*[218] have discussed the mechanism for the rearrangements of acyclic systems using $BF_3.OEt_2$ and concluded that there was a

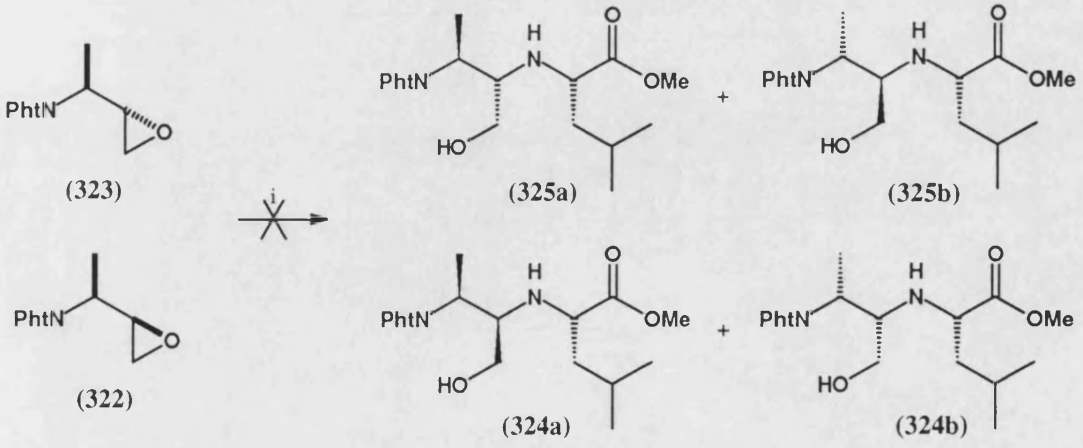
carbocation formed, which was then trapped as the fluoride intermediate before the rearrangements occur to give the corresponding carbonyl compounds (**scheme 81**).




i. $BF_3.OEt_2$, DCM.

**Scheme 81**

Our intentions were to establish a rapid entry into the reduced hydroxymethylene dipeptides (**324**) and (**325**) *via* the ring opening addition of Leu-OMe at the most substituted site of the homochiral epoxides (**322**) and (**323**). Our first attempt involved addition of the hydrochloride amine salt of Leu-OMe to the mixture of racemic epoxides (**322**) and (**323**), (4:6). We hoped that the hydrochloride would catalyse formation of a carbocation and then nucleophilic attack would take place, followed by treatment with base to afford the desired dipeptides (**324**) and (**325**). (**Figure 40**)




i. $BF_3.OEt_2$, DCM, 20°C, Leu.OMe.

**Figure 40**

The Leu-OMe.HCl (**395**) was produced by reacting hydrogen chloride gas with leucine in methanol. The hydrochloride amino ester (**395**) was formed in 96% yield. It is also commercially available. When formation of the dipeptides (**324**) and (**325**) was attempted using ester (**395**), no reaction occurred and only the starting materials were isolated. This reaction was repeated using the more basic and nucleophilic free amino ester (**396**) (prepared by treatment of Leu-OMe.HCl with aqueous ammonia in 93% yield) and boron trifluoride etherate to activate the epoxides (**322**) and (**323**). These conditions involved the addition of the boron trifluoride etherate to Leu-OMe and then addition of the epoxides to this complex. Unfortunately no coupling occurred. Instead the epoxides were converted to the corresponding diols (**338:337**, 1:3) (37%) and the $BF_3$-Leu-OMe (**397**) complex was also isolated after chromatography as identified from mass spectroscopy and $^1H$ n.m.r. data; [$\delta_H$ 0.96 (6H, $Me_2$), 1.7-1.8 (3H, $CH_2CH$), 3.8 (3H, OMe), 4.1 (1H, $CHCO_2Me$), 6.45 ($^+NH_3$); m/z (C.I.) 214 (4%, $M^+$+1)], **Figure 41**.


(396) (397)

i. $BF_3.OEt_2$, DCM.

**Figure 41**

As these conditions resulted in the complexation of the Lewis acid and amino ester, it was considered that addition of the Lewis acid to the epoxide first would overcome this problem. This procedure produced a white precipitate which immediately disappeared on the addition of the free amino ester (**396**). T.l.c. visualisation of the reaction mixture showed three new spots when viewed under short wavelength light. Isolation of the compounds and spectroscopic analysis confirmed that the coupling had occurred. Also observed were the diols (**337**) and (**338**). Analysis of the first spot,

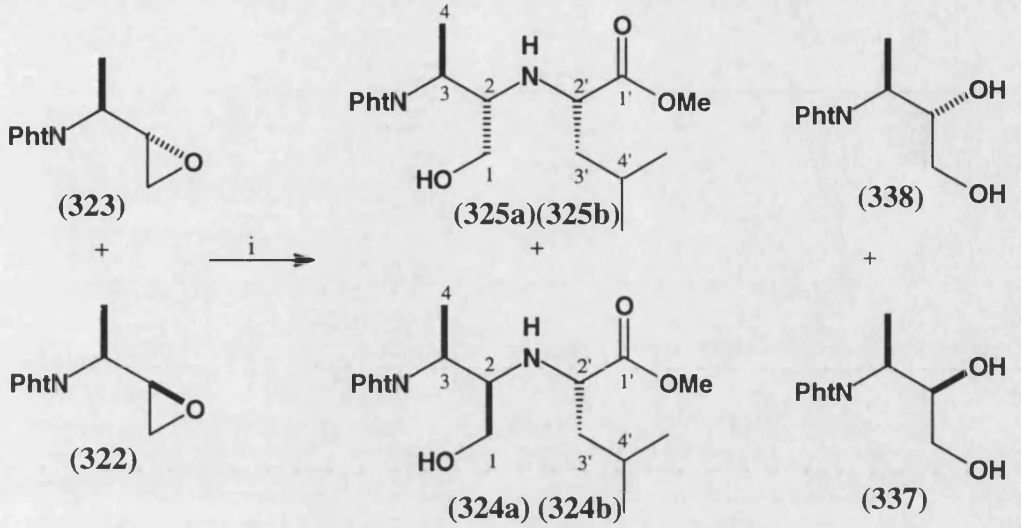
showed it to be the diols **(338)** and **(337)** in a 39:51 ratio. The second spot was found to be three compounds in a ratio of 1:3:6, *i.e.* **(338)** and **(337)** with dipeptide **(324b)** and the third spot ($R_f$ 0.41) to be the desired dipeptide **(324a)** **(Figure 42)**. **Table 10** shows the $^1H$ n.m.r. data for both the dipeptide mimetics **(324a)** and **(324b)**. Isomers **(325)** were not isolated.

**Table 10**

| Proton number | Isomer **(324b)** (*2S,3R,2'S*) $\delta_H$ ppm, *J* Hz | Isomer **(324a)** (*2R,3S,2'S*) $\delta_H$ ppm, *J* Hz |
|---|---|---|
| PhtN | 7.92 | 7.90 |
| PhtN | 7.72 | 7.70 |
| 3 | 4.97, qd 7.1, 3.9 | 4.87, qd 7.1, 4.4 |
| 2 | 3.85-3.96 | 3.89-3.95 |
| 1 | 3.40-3.53 | 3.49, d 6.4<br>3.50, d 5.2 |
| 2' | 5.09, dd 7.3, 6.4 | 5.08, dd 7.9, 6.0 |
| 3' | 1.90-1.96 | 1.90-1.99 |
| 4' | 1.66-1.80 | 1.70-1.87 |
| $Me_{Ala}$ | 1.54, d 7.1 | 1.52, d 7.1 |
| OMe | 3.77 | 3.74 |
| $Me_{Leu}$<br>$Me_{Leu}$ | 1.03, d 6.6<br>0.91, d 6.6 | 1.03, d 6.4<br>0.91, d 6.4 |

Analysis of the spectroscopic data confirmed the presence of the NH and OH protons in both the IR and the n.m.r. spectra, for both the hydroxymethyl dipeptides. IR and n.m.r. also showed that the leucine methyl ester was present *i.e.*, C=O stretch at 1734 $cm^{-1}$ and the 6H integral signal at 0.8-0.9 ppm for the methyl groups. Mass spectroscopy showed the mass ion for the dipeptide at 362 a.m.u. in the E.I. and the $(M^+ + 1)$ at 363 in the C.I. spectra. The proton n.m.r. spectra run in *d*-DMSO showed that the hydroxyl group was primary, thus eliminating the possibility that the product

had been formed *via* path A, (**Figure 39**). This useful technique owes its usefulness




i. $BF_3.OEt_2$, DCM.

**Figure 42**

to the fact that the DMSO complexes with the hydroxyl group preventing rapid exchange with the solvent. This has the effect of sharpening the broad peaks thus showing any splitting from adjacent protons. The stereochemistry for the major isomer **(324)** was not obvious. For mechanistic considerations (see Section **2.5.4**), it can be concluded that epoxide ring-opening reactions using $BF_3.OEt_2$ give diols with the major isomer of stereochemical configuration (2*R*,3*S*), independent of the epoxide(s) used. Indeed, in this case the major diol produced was the isomer **(337)** *i.e.* stereochemistry (2*R*,3*S*). It therefore may follow that the major dipeptide isolated should have the stereochemistry (2*R*,3*S*,2'*S*), *via* a double inversion of configuration at centre C-3. The first inversion involves participation of a phthaloyl carbonyl producing a 6,5,5 tricycle, which is then ring-opened at C-3 to give the dipeptide, with overall retention of configuration.

On closer inspection of the proton n.m.r. for the major isomer it was observed that the coupling constant between the protons on C-2 and C-3 was 4.3Hz. 2D Cosy analysis of the dipeptide **(324)** produced an inconclusive result; this suggested free rotation

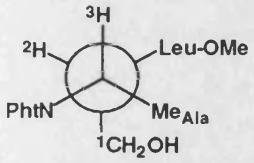
about this bond. As this was the case, a 2D nOesy experiment was performed so that we could build a 3D picture of this dipeptide. Assuming that the stereochemistry could be either (2*R*,3*S*,2'*S*), (2*S*,3*R*,2'*S*) or (2*S*,3*S*,2'*S*), (2*R*,3*R*,2'*S*), then studying all the possible interactions when the dihedral angle between these protons matches that derived from Karplus equation J=kcosθ, where θ = 115 and 65° in this instance. And then comparison with the interactions seen with the 2D nOesy spectra, will allow us to deduce the stereochemistry of this isomer. **Table 11** shows the through space interactions seen in the 2D nOesy spectra. Represented over are the five possible Newman projections for each isomer.

**Table 11**

| Observed proton | Interaction | Intensity |
|---|---|---|
| 3-H | $^{4}Me_{Ala}$ | very strong |
| 3-H | 2-H | moderate |
| 3-H | 1-H | weak |
| 2-H | $^{4}Me_{Ala}$ | moderate |
| 2-H | 1-H | very strong |
| 1-H | $^{4}Me_{Ala}$ | no interaction |

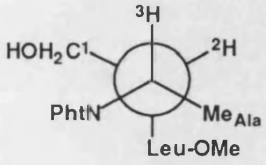
The only possible conformation allowed for the data collected is that for the (2*R*,3*S*, 2'*S*) or (2*S*,3*R*,2'*S*) isomers, where the protons 2-H and 3-H, the groups $^{1}CH_2OH$ and PhtN, and $Me_{Ala}$ and Leu-OMe all lie gauche to each other. The fact that 2-H and 3-H are gauche to each other is in accordance with the coupling constant. There is also a gauche interaction between the 2-H and $^{4}Me_{Ala}$ which fits the 2D nOesy spectra perfectly. It is also interesting to note that for all the products isolated with the stereochemical configuration (2*R*,3*S*) the chemical shift for 3-H was always lower than the corresponding isomer (2*S*,3*S*), see **Table 12**.

Isomer (**324**) (2*R*,3*S*,2'*S*), (2*S*,3*R*,2'*S*)



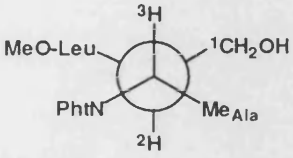

θ = 60°, *J* = 4Hz

1-H-$^{4}$Me$_{Ala}$ inter-action therefore not possible




θ = 60°, *J* = 4Hz

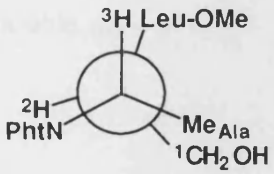
**excellent fit** with nOesy spectra




θ = 180°, *J*>10Hz

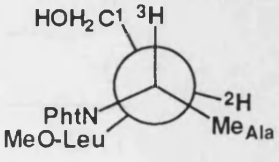
not possible, does not fit cosy

not possible




θ = 115°, *J* = 4Hz

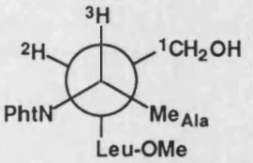
1-H-$^{4}$Me$_{Ala}$ inter-action therefore not possible




θ = 115°, *J* = 4Hz

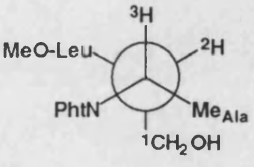
1-H and3-H inter-action would be strong, not possible

Isomer (**325**) (2*S*,3*S*,2'*S*), (2*R*,3*R*,2'*S*).



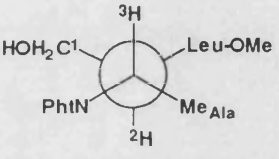

θ = 60°, *J* = 4Hz

1-H-$^{4}$Me$_{Ala}$ inter-action therefore not possible




θ = 60°, *J* = 4Hz

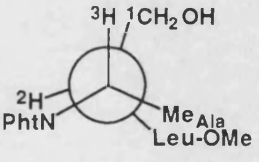
1-H-$^{4}$Me$_{Ala}$ inter-action therefore not possible




θ = 180°, *J*>10Hz

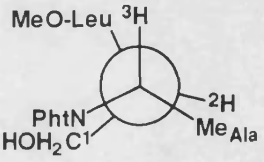
not possible, does not fit cosy

not possible




θ = 115°, *J* = 4Hz

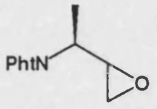
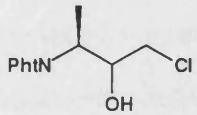
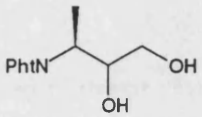
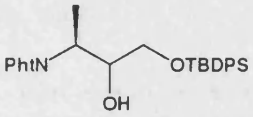
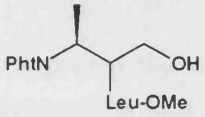
1-H-$^{4}$Me$_{Ala}$, 2-H interactions does not fit, therefore not possible




θ = 115°, *J* = 4Hz

1-H-$^{4}$Me$_{Ala}$ inter-action therefore not possible

**Table 12**

| Compound | isomer (2*S*,3*S*) 3-H (ppm) | isomer (2*R*,3*S*) 3-H (ppm) |
|---|---|---|
|  | 4.07 | 3.97 |
|  | 4.71 | 4.68 |
|  | 4.53 | 4.42 |
|  | 4.65 | 4.64 |
|  | 4.67 | 4.57 |

As this addition worked, the effect of temperature on the reaction was investigated. These results are detailed in **Table 13**. It is interesting to note that only the isomers (**324**) were isolated, independent of the starting material used, thus suggesting that a double inversion process was involved.

**Table 13**

| | % Yield isolated | | | | |
|---|---|---|---|---|---|
| Temperature/°C | Isomer used | amount recovered | diols (**338** and **337**) | diols (**338** & **337**) + dipeptide (**324b**) | (**324a**) |
| 20 | MIX | N/A | 2 (56:44) | 2 (2:8) 8 | 12 |
| 0 | (323) | 2.2 | 3 | 11 | 15 |
| -20 (1 hr) | (322) | 35 | 0 | 3 (2:8) 7 | 27 |
| -20 (2 hrs) | (322) | N/A | N/A | 5 | 8* |

* decomposed on silica

It was found that on long, narrow chromatographic columns the dipeptides decomposed with only 30% isolation of the products. Short, wide columns were used to achieve the greatest recovery, but unfortunately some product was always lost. With use of neutral alumina, the dipeptides adhered to the column, and were only removed by methanol. All flash chromatographic attempts at separating the diols from the hydroxymethyl dipeptide (**324b**) failed. Attempts at separating these compounds using HPLC apparatus resulted in cleavage of the newly formed C-N bond to give Leu-OMe and the corresponding diol (**337**). This was possibly caused by the trifluoroacetic acid protonating the secondary amine, thus allowing for displacement of Leu-OMe by water.

With all the previous epoxide opening reactions, the work ups involved dilute acid washes. We repeated the racemic epoxide opening reaction using 2 equivalents of Leu-OMe and one equivalent of $BF_3.OEt_2$ at -78°C and concentrated the crude mixture *in vacuo* ready for isolation of the products by chromatography. T.l.c. of the crude reaction mixture showed that some of the racemic epoxide (**323**) had not reacted and that the racemic diols (**337**) and (**338**) had been formed, due to hydrolysis by water. A new compound, previously not isolated before, with a lower retention factor than the desired dipeptide product, had also been produced. After spectroscopic elucidation the structure appeared to be that of the aziridine (**328**). There were no NH and OH peaks in the IR spectra, the n.m.r. spectra showed a shift upfield for the 1-H protons, to 2.40 and 2.77ppm, which indicates that they are deshielded to a lesser extent than those adjacent to an oxygen functionality. The coupling values for the protons 1B-H with 2-H are constituent for eclipsed protons , $J$ = 7.6Hz, dihedral angle of 0°, and 1A-H with 2-H where $J$ = 5.0Hz, for a dihedral angle of 120°. The rest of the spectra is consistent for that of the PthN-Ala-Leu-OMe dipeptide. The conditions must have allowed for the formation of the hydroxymethyl dipeptide (**324**) *via* ring opening, with the $BF_3$ having remained bound to the oxygen to make it a much better leaving group. The use of excess Leu-OMe could have acted as a base allowing the

more nucleophilic secondary amine to cyclise intramolecularly to give the aziridine (**328**). The secondary amine has essentially the same basicity as that of Leu-OMe, but because it lies in such a close proximity to the good leaving group its rate of attack is much higher (**Figure 43**).

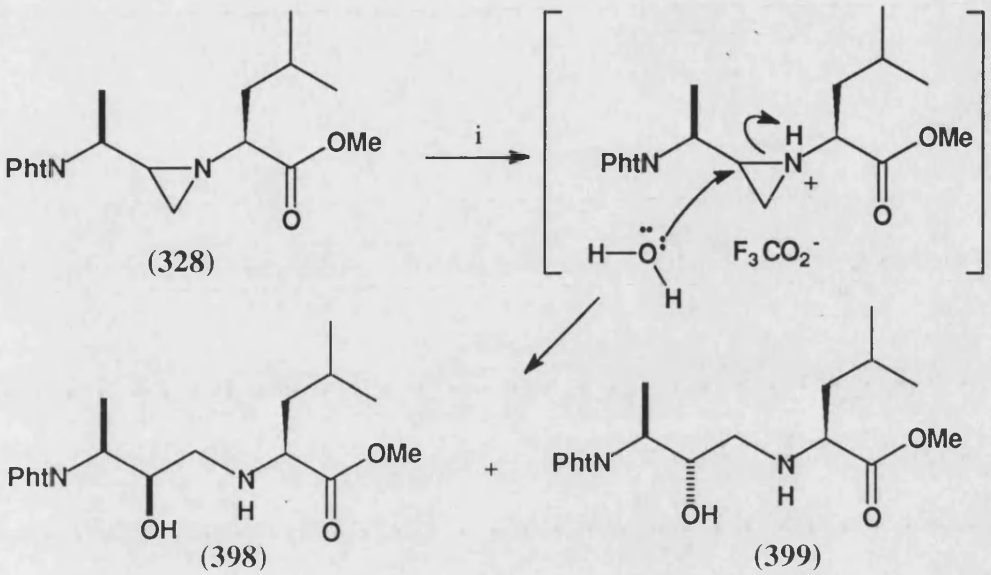

(328)

**Figure 43**

Further purification of the mixture of aziridines (**328**) was attempted using HPLC apparatus. Again, the solvent system used altered the structure of the product. Mass spectroscopy showed the new compound that had its molecular weight increased 18 a.m.u. (363 in FAB+) and it could be clearly seen in the proton n.m.r. and IR spectra that two new broad peaks corresponding to OH and NH were present. The chemical shifts of the $CH_2$ unit next to the hetero atom in these dipeptides (**398**) and (**399**) were markedly different to those of the previously formed hydroxymethyl dipeptides (**324**). This suggested that the hetero atom was nitrogen as opposed to oxygen according to previous data on the chemical shifts for such systems, (**Scheme 82**).

As nitrogen is less electronegative than oxygen, α-protons will be deshielded to a comparatively lesser extent than those *alpha* to oxygen. Indeed, the chemical shift for the $CH_x$-N are in the range 2.3-3.4 ppm and for oxygen they are at 3.4-3.8 ppm, the observed value was 2.85-3.4 ppm. The addition of the water to the aziridines (**328**) must have been at the most substituted site, *via* a $S_N1$ mechanism. The TFA most probably activated the aziridine (**328**) producing the secondary carbocation which was trapped by water to give the hydroxyethylene dipeptides (**398**) and (**399**). This is a

new route for the formation of the hydroxyethylamine dipeptide mimetics, not previously reported.

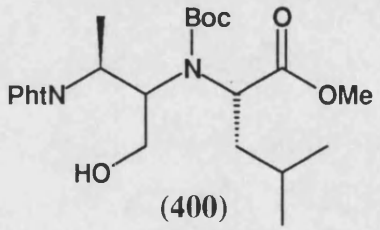



(328) (398) (399)

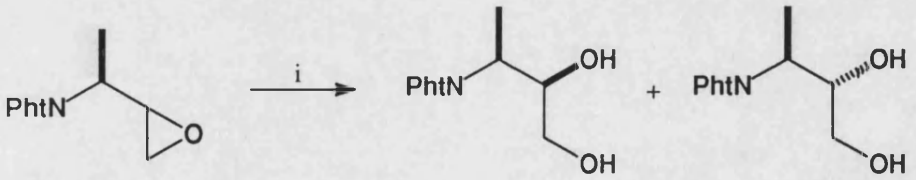
i. acetonitrile, water, TFA, HPLC.

**Scheme 82**

Another attempt to overcome this problem was to prepare the *N*-Boc protected dipeptide (**400**) derivatives, which would hopefully allow for separation of the desired product and by-products due to the increased lipophilicity of the dipeptide. A mixture of the *N*-phthaloylamino epoxides (**322**) and (**323**) were reacted with Boc-Leu-OMe in DCM with $BF_3.OEt_2$ as a Lewis acid (**Figure 44**).




(400)



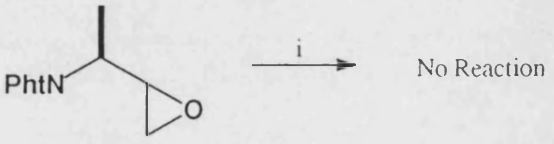

(322,323) (338) (337)

i. $BF_3.OEt_2$, Boc-Leu-OMe, DCM.

**Figure 44**

Addition of the amino ester did not occur. Instead the diols **(338)** and **(337)** were isolated in 69% yield (91:9 2*R*,3*S* as the major isomer, the absolute stereochemistry was established via protection of the minor isomer, to give the TBDPS derivative, X-ray crystallography showed it to be of the 2*S*,3*S* configuration).

One final attempt to make the hydroxymethyl dipeptides **(324)** and **(325)** (similar to the use of Boc-Leu-OMe) without contamination by the diols **(337)** and **(338)**, was to use the mono benzyl protected leucine methyl ester **(367)**. This protected amino ester was prepared by refluxing Leu-OMe.HCl with one equivalent of BnBr, $K_2CO_3$ in THF:EtOH (10:1) for 1 day. The product was isolated in 57% yield. The reaction was tried at -20°C and the work up involved a water wash. No hydroxymethyl dipeptides **(324)** and **(325)** were isolated, only the diols **(337)** and **(338)** and starting material being found. The steric bulk of the protected amino ester may have inhibited the reaction. Following these results no further *N*-phthaloylamino epoxide **(322)** and **(323)** ring opening reactions using amino esters and $BF_3.OEt_2$ were attempted, **Figure 45**.




**(322,323)**

i. $BF_3.OEt_2$, Boc-Leu-OMe, DCM.

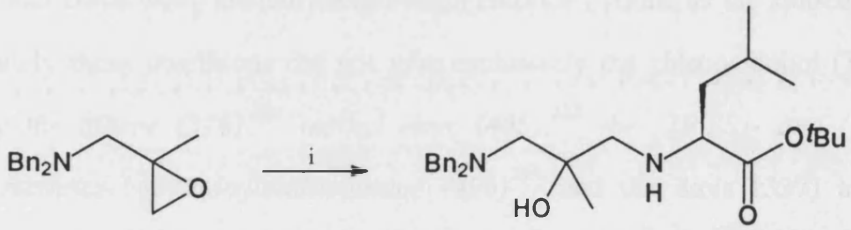
**Figure 45**

*Preparation of the N,N-dibenzyl protected hydroxymethyl dipeptide* **(401)**

The attempted preparation of the *N,N*-dibenzyl protected hydroxymethyl dipeptides **(401)** involved conditions analogous to those employed for the *N*-phthaloylamino protected epoxide ring opening by Bn-Leu-OMe. However, the unprotected amino ester was used **(396)**. The reaction when viewed by t.l.c. showed a huge number of compounds and we decided to abandon this protected route and go back to using the phthaloyl group.

*Preparation of the N,N-dibenzyl protected hydroxyethylamine dipeptides* **(402)**

Whilst preparing homochiral *N,N*-dibenzyl protected aminoepoxides **(356)** and **(357)** we found that isomerisation occurred during the Swern oxidation. This gave us the a mixture of the rearranged epoxides **(363)** and the expected epoxides **(356)** and **(357)**. The inseparable rearranged adducts **(363)**, were ring-opened following the procedure of Gordon *et al.*[124e] This gave us the expected hydroxyethylamine dipeptides **(402)** as an inseparable mixture in low yield (20%). These analogues were considered useful for biological screening and have been submitted for testing, (**Figure 46**).




(363) (402)

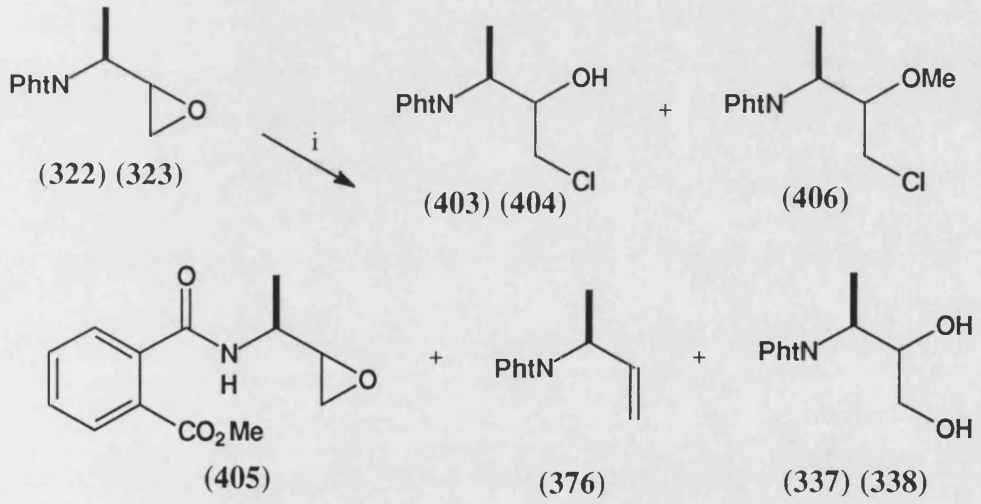
i. Leu-O$^t$Bu, MeOH, $Et_3N$, reflux, 20%.

**Figure 46**

### 2.5.2 Chloroalcohols

*Preparation of the N-phthaloylamino chloroalcohols* (**403**) *and* (**404**)

Epoxide ring-opening by nucleophilic addition of chloride has been widely explored. Many of the reported methods showed that the addition was not confined to the least hindered site, with addition also occurring at the more substituted site of the epoxide.[219,220] Gorzynski-Smith[222] illustrated that epoxides could be converted to the corresponding chloroalcohols under mild conditions with regioselectively, giving exclusively A2. We adopted these conditions, which involved refluxing the epoxide in methanol and DME using tetraethylammonium chloride hydrate as the source of "$Cl^-$". Unfortunately these conditions did not give exclusively the chloroalcohol (35%). In our study *the alkene* ***(376)***,[221] *methyl ester* ***(405)***,[222] *the (2R,3S)- and (2S,3S)-1-chloro-2-methoxy-3-phthaloylaminobutane* ***(406)***[223] and the diols (**337**) and (**338**) (16%) were also isolated. (**Figure 47**)



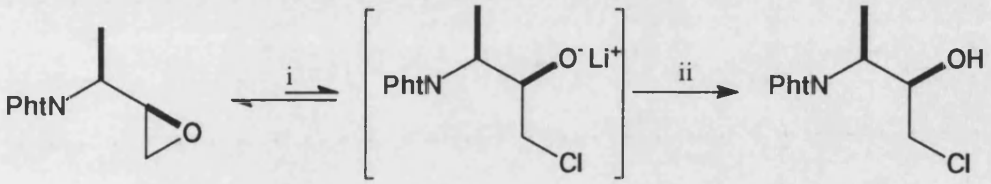

i. $Et_4NCl$, MeOH, DME, reflux, 1 day.

**Figure 47**

However, it is interesting to note that recently Suh *et al*[224] had detailed the use of tetraethylammonium bromide with the assistance of $Mg(NO_3)_2$ as an excellent Lewis acid catalyst. Here, the bromoalcohols were isolated typically in 78-97% yield with the corresponding epoxides cleaved regio and stereoselectively under neutral conditions.

The $Mg(NO_3)_2$ activates the epoxide so that attack only occurs at the less hindered carbon of the unsymmetrical epoxide.

Bajwa *et al*[225] reported that epoxides could efficiently be converted to the corresponding chloroalcohols using lithium chloride with acetic acid in THF. The addition goes regioselectively and in high yield, with retention of configuration (**Scheme 83**). Ring-opening of the recrystallised amino epoxide (**322**) using the procedure detailed by Bajwa, gave the amino chloroalcohol (**404**) quantitatively.




(322) (404)

i. LiCl, THF, RT; ii. AcOH.

**Scheme 83**

*Preparation of the dibenzylaminochloroalcohol* (**407**)

The *N,N*-dibenzyl aminoepoxide (**356**) and (**357**) were converted uneventfully to the corresponding chloroalcohol (**407**) in quantitative yield as outlined by Bajwa *et al*,[225] **Figure 48**. More recently Barluenga *et al*[226] have synthesised these compounds *via* reduction of the corresponding chloroketone, but they did not give any spectroscopic data.


i. LiCl, THF, AcOH, RT, 1 day, 70-100%; P = PhtN and $Bn_2$-

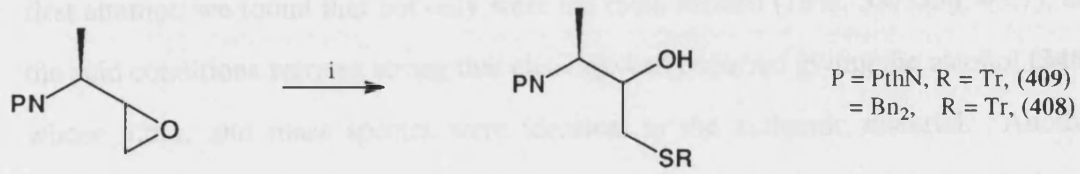
**Figure 48**

### 2.5.3 Thiol derivative

*Preparation of the (2S,3S)- and (2R,3S)-3-N-protected amino 1-(protected thiol)-2-hydroxybutanes*

The formation of the thiolmethyl dipeptide was also considered to be an important target. Both the *N*-protected epoxides (**322,323** and **356,357**) were ring-opened with triphenylmethyl thiol in methanol with triethylamine following a procedure reported by Corey *et al.*[227] Dellaria *et al*[228] and Luly *et al*[229] also employed this methodology to synthesis renin inhibitors using HSR, where R=$C_6H_{11}$ and isopropyl.

In our study the *N,N*-dibenzyl amino alcohol (**408**) was isolated in 9% yield and the *N*-phthaloylamino epoxides (**322**) and (**323**) were converted in 90% yield to the alcohols (**409**). The *N,N*-dibenzyl amino epoxides (**356**) and (**357**) were converted more efficiently to the corresponding protected thiol alcohols using 4-methoxyphenylmethyl-thiol (97% yield), **Figure 49.**



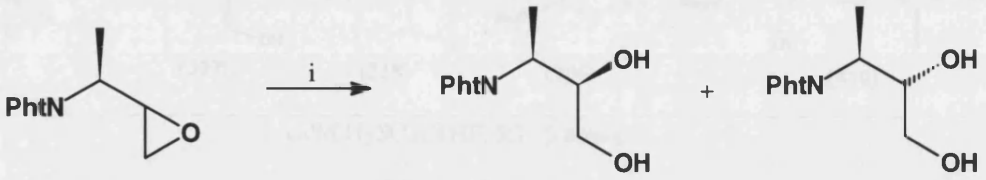

i. a) P=$Bn_2$ and PhtN, $HSCPh_3$, $Et_3N$, MeOH; b) P=$Bn_2$, *p*-methoxyphenylmethylthiol, $Et_3N$, MeOH.

**Figure 49**

### 2.5.4 Diol formation

*Preparation of the phthaloylamino dihydroxybutane via epoxide ring-opening*

In the course of the synthesis of the hydroxymethyldipeptides (**324**) and (**325**) from the epoxides (**322**) and (**323**), using the Lewis acid $BF_3.OEt_2$, we found that the diols (**337**) and (**338**) were generally formed in modest yield as the unwanted by-products (69% when Boc-Leu-OMe was used see Section **2.5.1**), **Figure 50**.



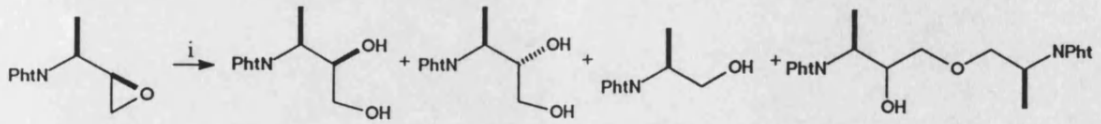

(322), (323) (337) (338)

i. a) $BF_3.OEt_2$, DCM, $H_2O$, -23°C; b) 0.1M $H_2SO_4$, THF, RT, 3 days; c) Dowex-50X, THF, $H_2O$, 50°C, 11 hrs or d) 6% $HClO_4$, THF, RT, 2 days.

**Figure 50**

We considered using $BF_3.OEt_2$ at lowered temperatures to activate the epoxides so that they would be hydroxylated by water to the corresponding diols **(337)** and **(338)**. Upon our first attempt at -78°C the diols **(337)** and **(338)** were generated in low yield (24%) with most of the starting material recovered. When we repeated the reaction at -20°C the yield was only increased to 35% in a ratio of 9:91 with the isomer of 2*R*,3*S* configuration being the major isomer **(337)**. We considered using the more common method of converting epoxides to diols, *i.e.* using a dilute acid such as $H_2SO_4$. At our first attempt, we found that not only were the diols formed (19%, **337:338**, 93:7), but the acid conditions were so strong that cleavage had occurred giving the alcohol **(346)**, whose n.m.r. and mass spectra were identical to the authentic material. Another product was isolated, the n.m.r. spectra suggested that the isolated product was almost symmetrical in nature and may have been formed *via* addition of the alcohol **(346)** to the starting material to give **(410)**. The proton n.m.r. showed three sets of aliphatic protons, in the ratio of (4:2:1). The four protons had chemical shifts expected for ethers, the two protons showed coupling signals to a methyl group and the remaining proton had the expected chemical shift, for a -C*H*(OH)- proton, however we did not known the stereochemistry for this centre, **Figure 51**.

We reduced the concentration of the acid to 0.1M $H_2SO_4$,[230] thus allowing successful conversion of the epoxides to the corresponding diols **(337)** and **(338)**, in moderate yield (54%, **337:338**, 91:9).




(322) (337) (338) (346) (410)

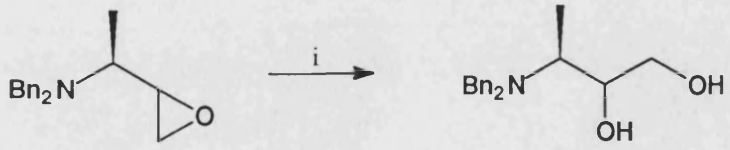
i. 1.0M $H_2SO_4$, THF, RT, 5 hours.

**Figure 51**

The yield was increased slightly when we employed the ion exchange resin Dowex-50X8-100 (strongly acidic), giving the diols (**337**) and (**338**) in 56% yield. We discovered the best way to produce the diols (**337**) and (**338**), was to use 6% $HClO_4$.[231] This gave the diols (**337**) and (**338**) in good yield (62-100%, **337:338**, 3:1), these results are shown in **Table 14**.

**Table 14**

| Epoxide ratio (**323:322**) | Acid catalyst | Yield (%) | Ratio of diols (**338:337**) |
|---|---|---|---|
| 43:57 | $BF_3.OEt_2$ | 69† | 9:91 |
| 43:57 | $BF_3.OEt_2$ | 35 | 9:91 |
| 43:57 | 0.1 $H_2SO_4$ | 54 | 9:91 |
| 0:100 | 1.0 $H_2SO_4$ | 19 | 7:93 |
| 0:100 | Dowex | 56 | N/A |
| 35:65 | $HClO_4$ | 62-100 | 1:3 |




(356) (357) (411)

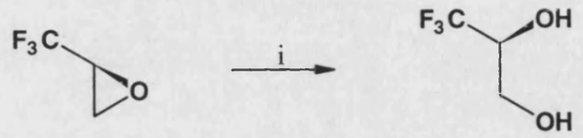
i. a. $BF_3.OEt_2$, -20°C, DCM; or b. Dowex-50, THF, RT.

**Figure 52**

† as the by-product of the ring-opening of the epoxides (**322**) and (**323**) by Boc-Leu-OMe

*Mechanism*

Katagiri *et al*[234] showed that the epoxide ring-opening of 1,2-epoxy-3,3,3-trifluoropropane **(414)** went with retention of configuration, when they used dilute $H_2SO_4$, following the procedure detailed by McBee.[235] This suggests that the opening went exclusively *via* a $S_N2$ mechanism, **Figure 55**.

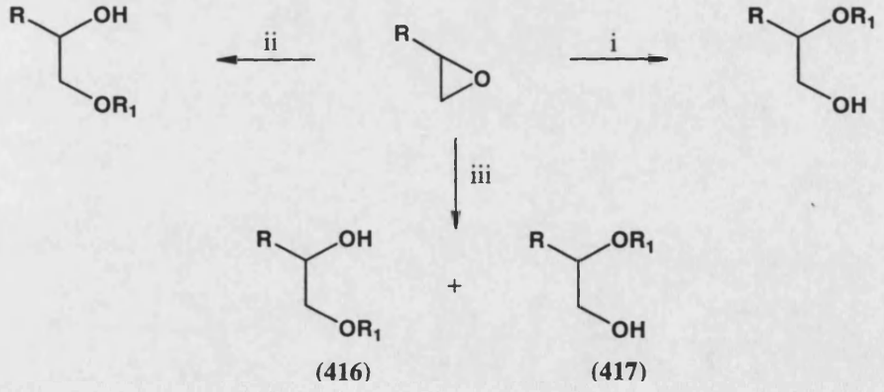



**(414)**, 75 % e.e. **(415)**, 75 % e.e.

i. $H_2SO_4$, $H_2O$, 80%.

**Figure 55**

Engel *et al*[236] reported that the nature of the substituents on epoxides had a degree of control over which mechanistic pathway when epoxides were opened using catalytic $BF_3.OEt_2$. They concluded that stabilising groups *e.g.* phenyl or allyl, gave exclusively addition at the substituted carbon *i.e.* an $S_N1$ pathway, whereas alkyl groups cause additions at the terminal carbon because of steric reasons *i.e.* $S_N2$ pathway, and groups which lay between those extremes gave a mixture of products, **Scheme 84**.



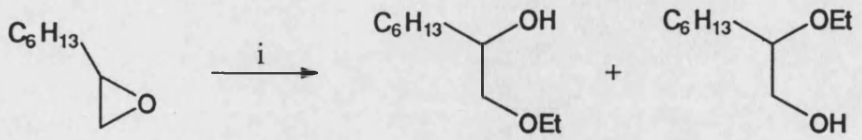

**(416)** **(417)**

**Scheme 84**

Schnurpfeil *et al*[237] had reported that $BF_3.OEt_2$ and other acids catalysed the ring-

Schnurpfeil *et al*[237] had reported that $BF_3.OEt_2$ and other acids catalysed the ring-

opening of 1,2-epoxyoctane **(418)** with ethanol, giving various mixtures of products, **Figure 56**, (**Table 15**).



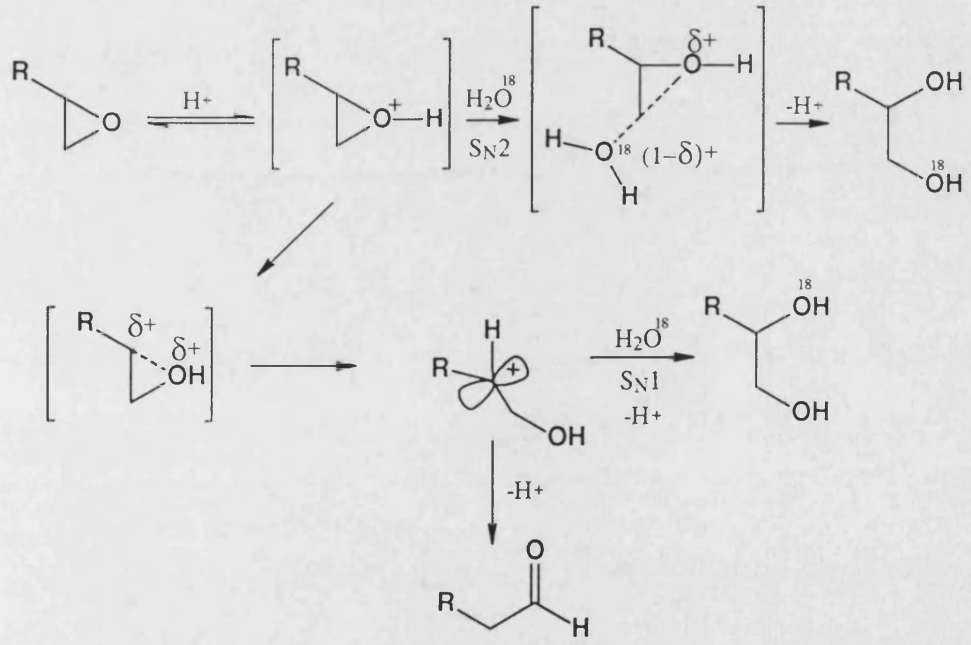

**(418)** **(419)** **(420)**

i. a) $BF_3.OEt_2$, EtOH; b) $HClO_4$, EtOH or c) *p*-TSA, EtOH.

**Figure 56**

**Table 15**

| catalyst | epoxide (**419**) % | epoxide (**420**) % |
|---|---|---|
| $HClO_4$ | 52.8 | 47.2 |
| $BF_3.OEt_2$ | 60.5 | 39.5 |
| *p*-TSA | 71.6 | 28.4 |




rearrangement product

**Scheme 85**

Pocker *et al*[238] carried out isotopically labelled water experiments to determine the mechanisms of epoxide ring-openings. They found from the acid catalysed ring-opening reaction, that the reaction could be interpreted in terms of a spontaneous

opening of the epoxide to generate a carbocation, followed by subsequent capture by water which leads to a vicinal diol (**Scheme 85**). However, the evidence for this is sparse and inconclusive.

They found that the rearrangement process, which was similar to the pinacolic rearrangement, could be facilitated by increasing the acidity of the media and by using polar, non-nucleophilic solvents. **Table 16** shows the amount of $S_N1$ and $S_N2$ observed in the acidic limb of their investigations.

**Table 16**

| epoxide | $S_N1$ (%) | $S_N2$ (%) |
|---|---|---|
| propylene | 70 | 30 |
| isobutylene | 99 | 1 |

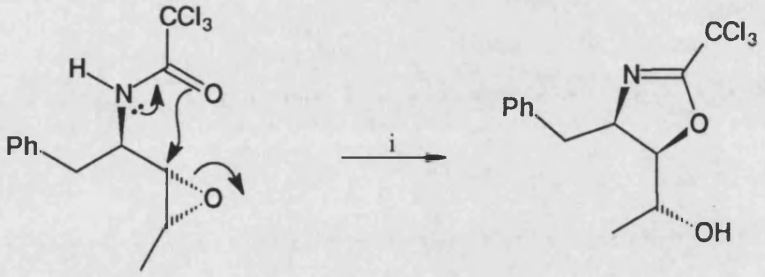
Long and Pritchard[239] in 1956 carried out similar work to Pocker. They explored the effect of $HClO_4$ at different concentrations on propylene and isobutylene, **Table 17**.

**Table 17**

| epoxide | $HClO_4$ (M) | $S_N1$ (%) | $S_N2$ (%) |
|---|---|---|---|
| propylene | 0.12 | 74 | 26 |
| propylene | 0.25 | 66 | 34 |
| isobutylene | 0.12 | 100 | 0 |
| isobutylene | 0.1 | 99 | 1 |

These reports did not study the stereochemical outcome of the $S_N1$ pathway. Our results, **Table 14**, were very similar to those of Pocker *et al*[238] and Long and Pritchard,[239] **Table 17**. These results were rather inconclusive and there was always a mixture of $S_N1$ and $S_N2$ addition. With the phthaloylamino protected epoxides (**322**) and (**323**), we found generally that there was a excess of one diastereomer formed,

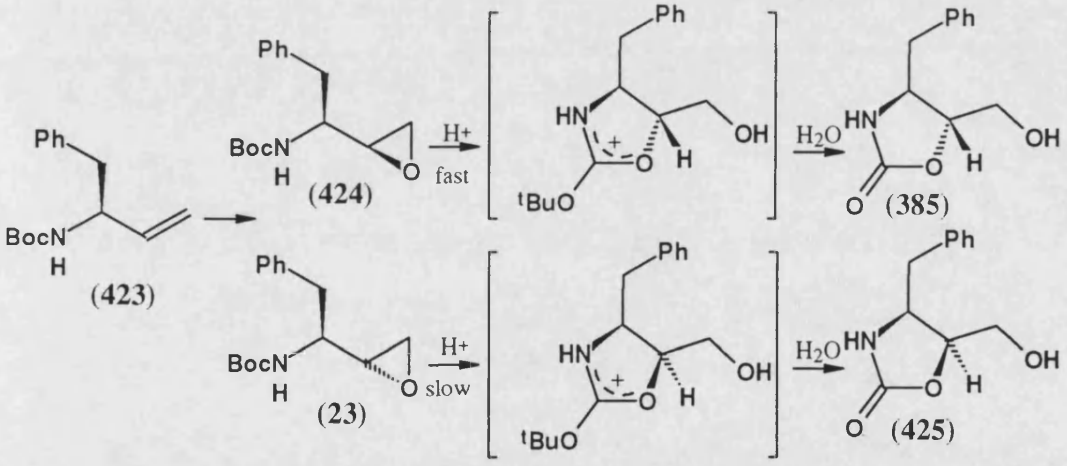
namely diol (**337**). This result was independent of the diastereomeric ratio of the epoxides (**322**) and (**323**) used. From these results, it may follow that the addition of water to the epoxide goes mainly *via* a $S_N1$ pathway, but it is unclear why there should be such an excess of the (2*R*,3*S*) stereochemistry. Thus, this suggests a more complex mechanism, which may involve the phthaloylamino protecting group. Roush *et al*[240] had described the formation of oxazolidine (**422**) *via* a 5-*exo*-trig cyclisation (**Figure 57**).




(421) (422)

i. amberlite resin.

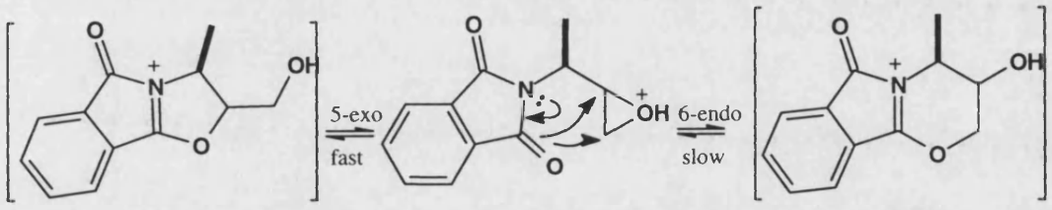
**Figure 57**




**Scheme 86**

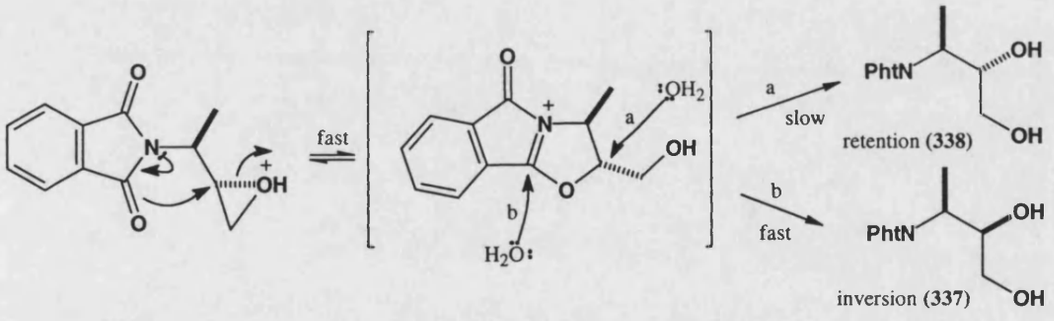
More recently Rich and Romeo[196] have reported the involvement of the Boc protecting group in the diastereomeric enrichment of the epoxidation reaction of (3*S*)-Boc-4-phenylbut-1-ene (423) with *m*-CPBA (**Scheme 86**). In this case the minor isomer (**424**) rearranges more rapidly to the oxazolidine (**385**), thus increasing the ratio for the major isomer (**23**).

The phthaloylamino group may be participating in a similar manner[241a] although the intermediates look rather ring-strained. The activation of the racemic epoxides under acidic conditions, followed by an intramolecular cyclisation *via* a 5-*exo*-trig addition, which will proceed faster than the 6-*endo*-trig cyclisation,[241b] will give the 6,5,5-tricycle system **(426)** and the corresponding mirror image (**Scheme 87**). The intramolecular cyclisation will be faster than the intermolecular addition of water. The 6,5,5-tricycle system **(426)** can then undergo attack by water to give the diols **(337)** and **(338)**, **Scheme 88**.



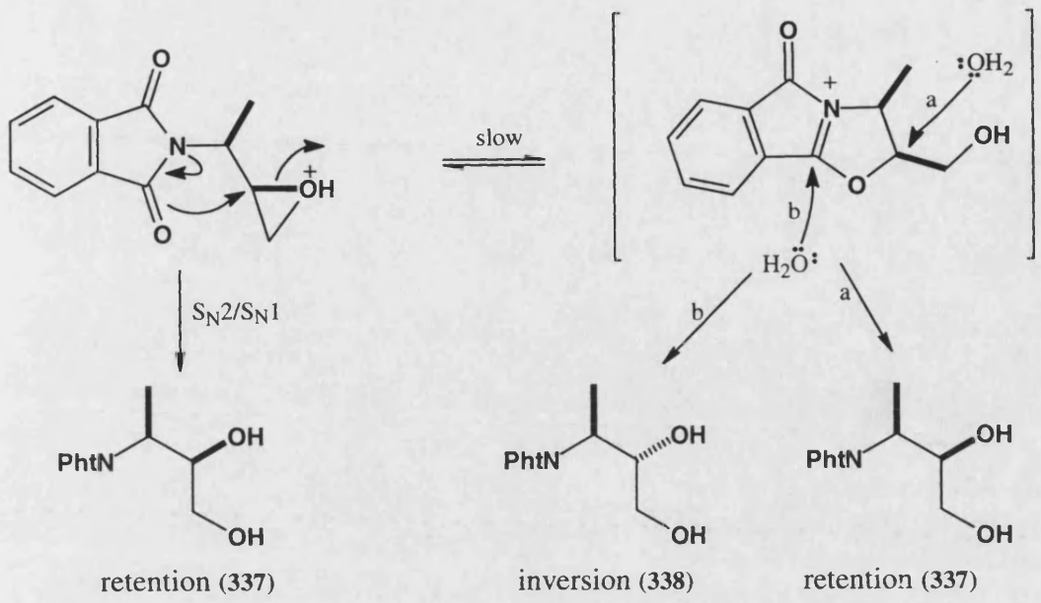

**(426)** **(427)**

**Scheme 87**




**Scheme 88**

The formation of the 6,5,5-tricycle system **(426)** may occur more rapidly for the minor isomer **(338)**, as the methyl and $CH_2OH$ groups will lie *trans* to each other. Addition of water to the imine will give inversion at C-2, giving the major diol **(337)**, **Scheme 88**, whereas, the major epoxide isomer may form slower, due to the methyl and $CH_2OH$ groups lying *cis* to each other. This steric factor may be sufficient enough to slow down the cyclisation process to allow intermolecular addition of water to compete. Thus the mechanism will follow $S_N2/S_N1$ pathways as detailed by Pocker *et al*[238] and Long *et al*,[239] producing diol **(337)** as the major diastereomer, **Scheme 89**.




**Scheme 89**

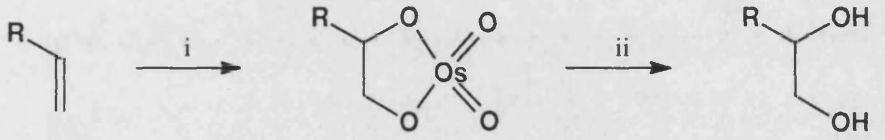
We did not carry out any isotopic labelled experiments which would have shown which mechanism may have taken place *i.e.* if there was any $^{18}O$ present in the phthaloylamino group (which could be removed with hydrazine), would suggest, formation of the 6,5,5-tricycle imine (**426**) which followed attack by water. Alternatively, if the was no $^{18}O$ present in the phthaloylamino portion, then this would show that the mechanism followed a straight forward $S_N2/S_N1$ pathway and involved no participation of the phthaloylamino group. There is still much debate about the mechanism of this epoxide ring-opening reaction, and that represented here maybe a possible route, but there may be other plausible mechanisms.

*Preparation of N-phthaloylamino dihydroxybutanes* (**337**) *and* (**338**) *via the hydroxylation of alkene* (**376**)

There are several oxidative reagents which are capable of converting alkenes to their corresponding diols. Potassium permanganate in aqueous solution has been widely used as an efficient oxidant in preparative chemistry. This technique had been further improved by the use of phase-transfer catalysts, *e.g.* $KMnO_4$-TEBAC[242] and $MnO_4$-CTMA.[243] Generally the diols were formed in good yields. However, over-oxidation

was occasionally a problem and this made us consider using another method which is also commonly used, namely hydroxylation using catalytic osmium tetroxide.[244-246]

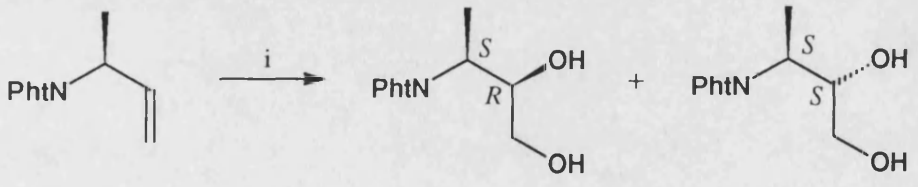
Ray and Matterson[244] reported that catalytic $OsO_4$ used with NMO proved highly successful as a preparative method for diols. These reactions went cleanly and in high yield. Ray and Matterson[244] also suggested that the intermediate osmate esters **(428)** could be hydrolysed quicker using pyridine to release the diols [when R=PhtNCH(Me) (**337**) and (**338**)], **Scheme 90**. When we followed their experimental details we only managed to produce diols (**337**) and (**338**), in moderate yield (44%), with the (2*S*,3*S*) isomer (**338**) as the major diastereomer (**337:338**, 27:73), **Figure 58**.




(428) (337) and (338)
R=PhtNCH(Me)

i. $OsO_4$, NMO, acetone; ii. pyridine.

**Scheme 90**




(376) (337) (338)

i. $OsO_4$, NMO, acetone:water:$^t$BuOH, (6:5:1ml), pyridine, 44%.

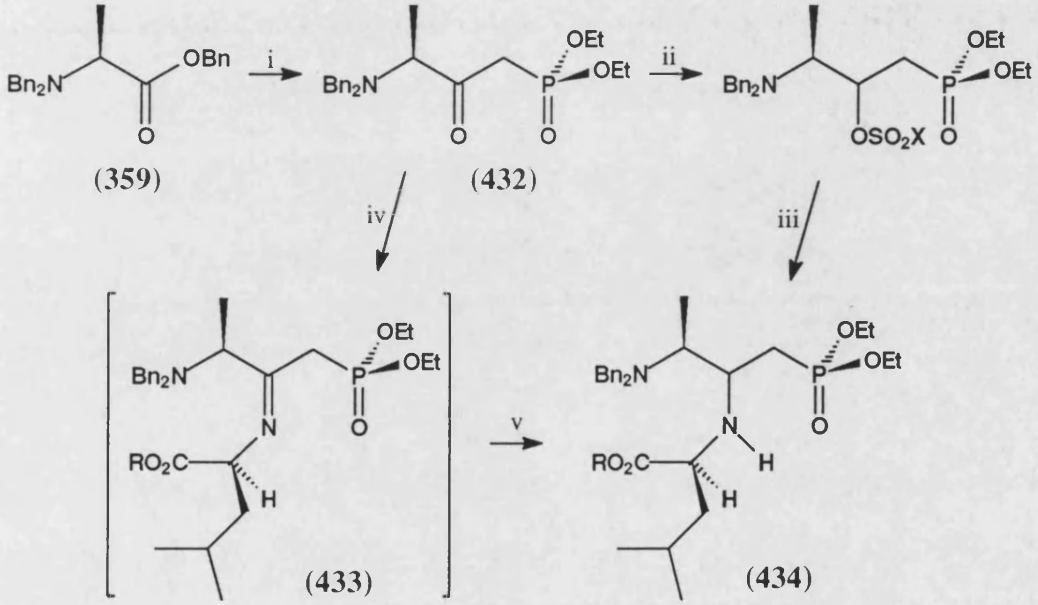
**Figure 58**

The fact that the major isomer produced using $OsO_4$ is the minor isomer in the epoxide ring-opening step, means that both isomers can be prepared in good yield from alanine.

## 2.6 Coupling reactions

### 2.6.1 Phosphonomethyl dipeptide (330)

The formation of a phosphonomethyl dipeptide analogue (**330**) was attempted using two possible paths (**Scheme 91**). The first path would involve the formation of the ketophosphonate (**432**), reduction and then activation of the alcohol followed by displacement with Leu-OMe to give the fully protected dipeptide (**434**). A second route would employ a reductive amination step, (**Scheme 91**).



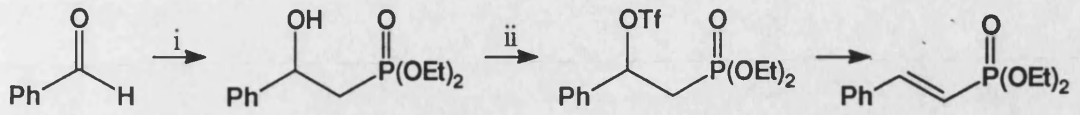

i. $CH_3PO(OEt)_2$, *n*-BuLi, -78°C, THF, 63-97%; ii. reduction, activation, X= toluene, $CF_3$ or Me; iii. coupling; iv. amination; v. reduction; R=Me.

**Scheme 91**

*Preparation of 1-(diethyl)phosphonate-2-phenyl-2-trifluorosulfonylethane* (**429**)

A model route was tried using benzaldehyde to see if the sulfonate could be eliminated under weak basic conditions. Benzaldehyde was treated with methyldiethylphosphon-

ate and *n*-BuLi at -78°C to yield the β-hydroxyphosphonate **(430)** in 30% yield, **Figure 59**.




**(430)** **(429)** **(431)**

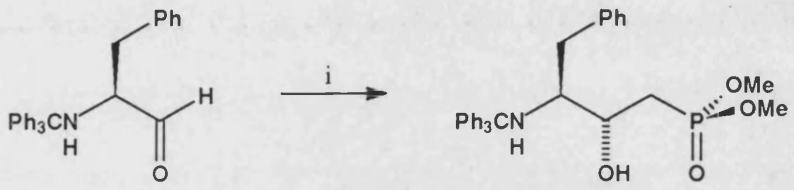
i. *n*-BuLi, methyldiethylphosphonate, -78°C, 30%; ii. Triflic anhydride, pyridine, DCM, -23°C, 25%.

**Figure 59**

Activation of the alcohol can be achieved in a manner of ways, preparation of the sulfonate being the simplest and most efficient method. The trifluoromethanesulfonate is the most reactive sulfonate,[247] about $5.6x10^4$ times a relatively better leaving group than the methanesulfonate moiety. The trifluoromethanesulfonates were first prepared by Leroux and Perkin[248] to form halides, and then Vedejs *et al*[249] in the following year, who used them to form C-S bonds. Many groups have prepared compounds containing C-N bonds using these sulfonates[247,250-253] and we sought to use them to prepare a new family of compounds. The trifluoromethanesulfonate can be prepared using either the trifluoromethanesulfonyl chloride or the trifluoromethanesulfonate anhydride. However, Just and Hakimelahi[254] reported that trifluoromethanesulfonyl chloride also acts as a mild chlorinating agent and therefore to avoid this problem we opted to use the anhydride and followed the procedure detailed by Effenberger *et al.*[250] The alcohol was treated with triflic anhydride and pyridine at -23°C for 1 hour. The major product isolated was the (*Z*)-alkene **(431)**, formed *via syn*-elimination of the trifluoromethanesulfonate group. The stereochemistry was proved by 2D nOesy n.m.r. spectra which showed that there where spatial interactions between the phenyl group and the phosphonate as well as there being a *cis* relationship for the two olefinic protons. 400 MHz proton n.m.r. spectra showed that the *J* value was higher than for normal *cis* alkenes, *i.e.* 2-8Hz, here the value was $J_{cis}$ 17-20Hz. Small amounts of the (<5%) of the desired sulfonate **(429)** were also observed by 400 MHz proton n.m.r.

Although we did not manage to isolate the sulfonate (**429**), we decided to take the alkene (**431**) and reflux it with Bn-Leu-OMe (**367**) to see if any 1,4-addition could be effected. Under these conditions, no addition occurred and only starting materials were isolated.

Although the corresponding sulfonate (**429**) of the β-hydroxyphosphonate (**430**) appeared to be unstable, the preparation of the *N,N*-dibenzyl protected aminophosphonate (**432**) was undertaken. Dellaria and Maki in 1986[255] prepared phosphostatine derivatives by reacting trityl protected amino aldehydes (**435**) with the lithium or sodium salt of methyl dimethylphosphonate to give the corresponding β-hydroxyphosphonate (**436**) (**Figure 60**), in good yield (67%).

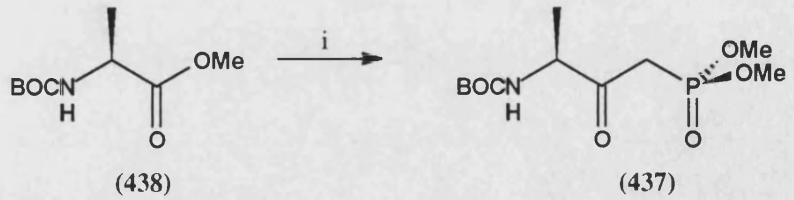



(435) (436)

i. Li or $NaCH_2PO(OMe)_2$, -78°C, THF, 67%, 3:1.

**Figure 60**

However, it was reported in 1987 by Chakravarty *et al*[256] that the β-ketophosphonates (**437**) could be generated in almost quantitative yield, when the ester (**438**) was reacted in the same manner, **Figure 61**.

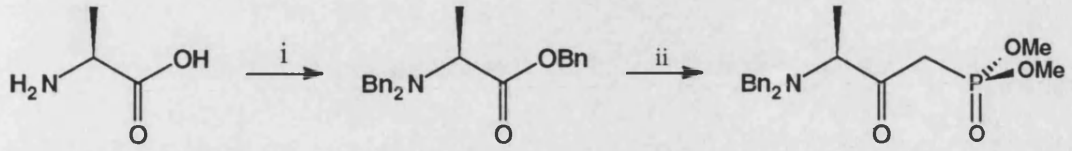



(438) (437)

i. $LiCH_2PO(OMe)_2$, -78°C, THF, 78%.

**Figure 61**

Alanine was protected by benzyl bromide as reported before,[165] in good yield. The *N,N*-dibenzylaminobenzylester (**359**) was reacted with the lithium anion of the methyldiethylphosphonate in THF at -78°C to give the desired product (**432**) in good yield (60% using 1.1 equivalents, with no epimerisation $\alpha_{589}$ = -67.5°). The yield was increased to 96% using 6 equivalents (with no epimerisation) following the procedure detailed by Chakravarty.[256] (**Figure 62**)

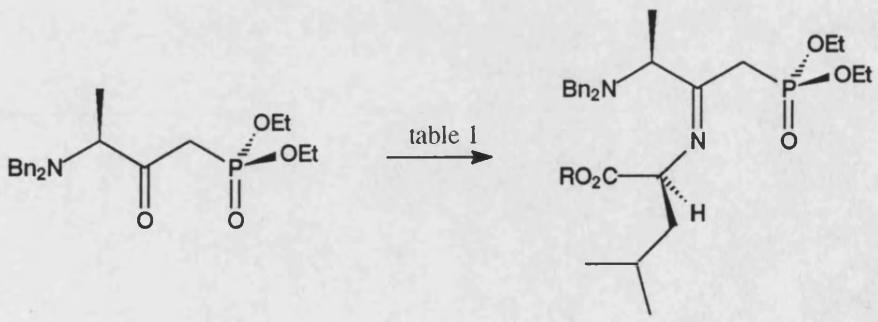



(359) (432)

i. BnBr, $K_2CO_3$, NaOH, EtOH, $H_2O$, reflux, 97%; ii. *n*-BuLi, THF, methyldiethylphosphonate, -78°C, 96%.

**Figure 62**

The first attempted preparation of the phosphonomethyl dipeptide (**330**) involved condensation[257] of the β-ketophosphonate (**359**) with Leu-OMe (see **Figure 63**), using a Dean-Stark distillation apparatus. This method did not give the desired product. The only material isolated, apart from starting material, appeared to be that of a leucine trimer, only the proton n.m.r. was run as we had no interest in this product. The imine (**433**) formed, probably decomposed or polymerised rapidly, as there are no stabilising groups attached to the nitrogen. Unfortunately other condensation reactions which used drying agents such as $TiCl_4$[258] or molecular sieves with *p*-TSA[259] also failed to give the desired product. See **Table 18**.




(432) (433)

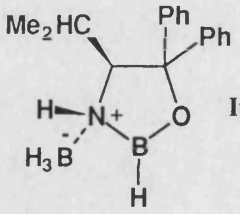
**Figure 63**

**Table 18**

| Amine | Reagents | Conditions | Time | Result |
|---|---|---|---|---|
| Leu-OMe.HCl | $Et_3N$, Toluene | Reflux | 6 hrs | generated new material, which appeared to be leucine trimer |
| Leu-OMe.HCl | *p*-TSA, Toluene | Reflux | 6 hrs | no dipeptide isolated |
| Leu-O$^t$Bu.HCl | $Et_3N$, $TiCl_4$ | Reflux | 2 days | no observed reaction |

Another successful method for the preparation of C-N bonds is *via* a reductive amination reaction, **Figure 64**. There is much literature precedent for reductive aminations, including the use of catalytic hydrogenation.

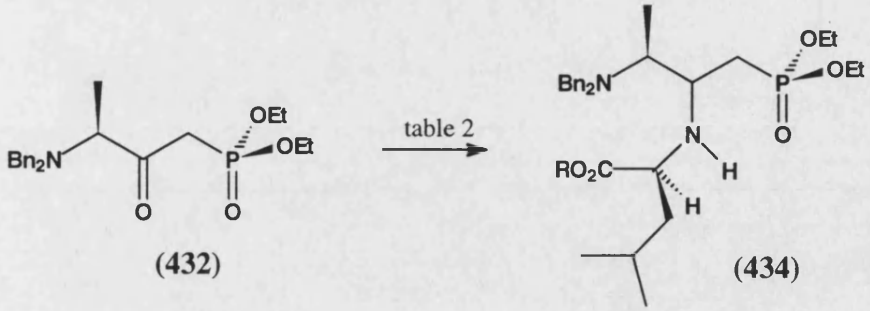
Other possible reducing agents include Itsuno's reagent (**439**),[260] sodium cyanoborohydride,[261] sodium triacetoxy borohydride[262] and iron pentacarbonyl with alcoholic KOH.[263]



Itsuno's reagent

**(439)**

Following the unsuccessful attempts at making the imine *via* a condensation reaction, we turned our attentions to the use of sodium cyanoborohydride as reported by Borch *et al.*[261] In our hands no coupling occurred and the only isolated product apart from starting material was the β-hydroxyphosphonate (**440**), **Table 19**. When the reductive amination was tried in acidic media (to activate the reduction step) again the β-hydroxyphosphonate (**440**) was isolated. This route was abandoned and efforts were concentrated on the route using the β-hydroxyphosphonate (**440**) as the precursor to the coupling step (see **Scheme 92**).



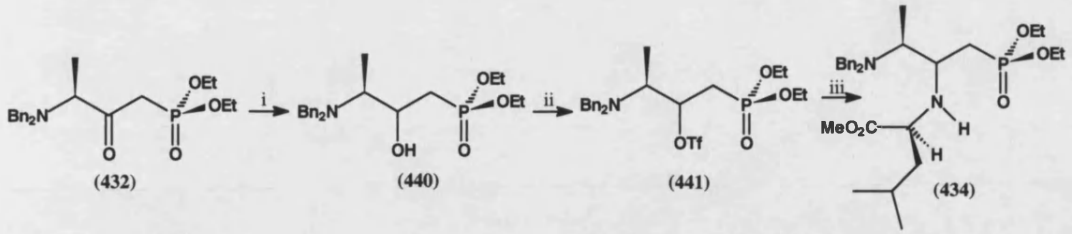

(432) (434)

**Figure 64**

**Table 19**

| Amine | Reagents | Conditions | Time | Result |
|---|---|---|---|---|
| Leu-OMe.HCl | $NaCNBH_3$ NaOAc, MeOH | RT | 2-3 weeks | reduced ketone to the hydroxyl, yield 80% |
| Leu-OMe.HCl | $NaCNBH_3$ NaOAc, MeOH | RT Reflux | 1 day 28 hrs | recovered starting material and the β-hydroxyphosphonate |
| Leu-OMe.HCl | $Na(OAc)_3BH$ DCE, AcOH $Et_3N$ | 50°C RT | 1 day 12 days | recovered starting material and the β-hydroxyphosphonate |

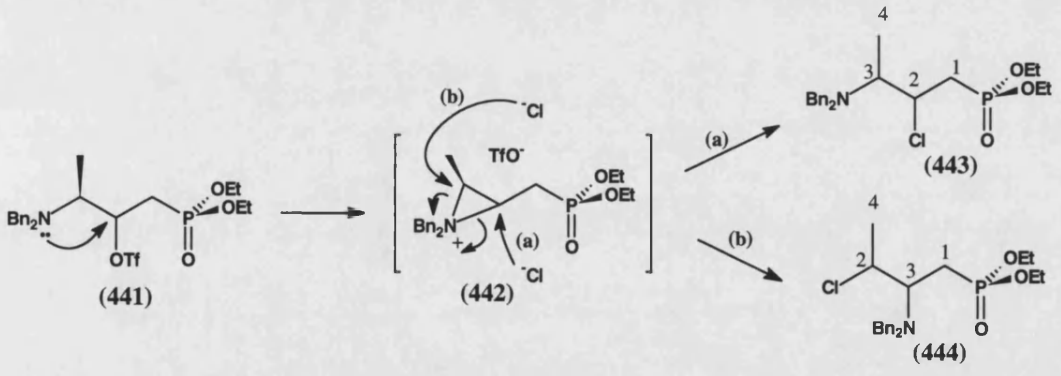
The β-ketophosphonate **(437)** was reduced using $NaBH_4$ to the corresponding alcohol **(440)**, an inseparable mixture of the (2*S*,3*S*) and (2*R*,3*S*) diastereoisomers in 95% yield. The β-ketophosphonyl sulfonate **(441)** was formed in an analogous manner to the other sulfonates, the product was not purified and the crude material was used in a model reaction, which employed the treatment of the sulfonate with Leu-OMe in THF. This did not afford the desired compound **(434)**. When this reaction was repeated in the presence of sodium acetate no reaction occurred, or when DMF was used as a solvent. (**Scheme 92**).




i. $NaBH_4$, EtOH, 95%; ii. Triflic anhydride, pyridine, DCM, -23°C; iii. a) Leu-OMe, THF, 0°C, 5 hrs, RT, 6 hrs; b) Leu-OMe, NaOAc, THF, RT, 3 days; c) Leu-OMe, THF, DMF, 0-RT, 3 days, remove THF, 2 hrs, no change.

**Scheme 92**

As the model reactions did not work, we repeated the preparation of the sulfonate **(441)** and characterised it fully. Spectroscopic studies showed that a rearrangement had occurred, $^{19}F$ n.m.r. spectra showed that there was no fluorine present. Proton and carbon n.m.r. spectra showed that the backbone had been modified and C.I. and E.I. mass spectroscopy showed the presence of chlorine. Previously, all other attempts at making trifluoromethanesulfonates of *N*-phthaloylamino alcohol derivatives had worked very well. We came to the conclusion that the trifluoromethanesulfonate had been generated, but had rearranged rapidly. One possible rearrangement could involve the nitrogen lone pair, displacing the trifluoromethanesulfonate to give the aziridinium trifluoromethanesulfonate salt **(442)**. This intermediate could then be converted into the chloro compounds **(443)** and **(444)**, (**Scheme 93**).

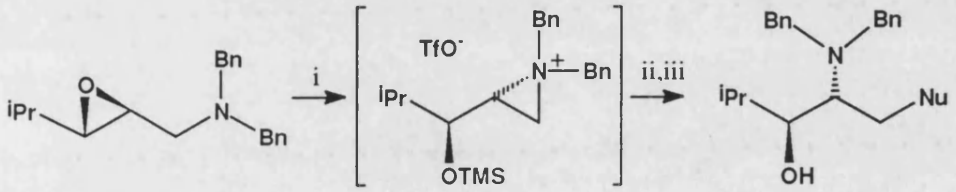



**Scheme 93**

After extensive spectroscopic analysis the γ-chlorophosphonate **(444)** was found to be the only compound generated, *via* Path (b), perhaps due to the steric hindrance of the

bulky phosphonate blocking approach *via* Path (a). However, following this pathway four possible diastereoisomers could be formed, and therefore it is not possible to comment on the stereochemistry here.

Rayner *et al*[170] had reported the existence of such salts. These had been generated by treating 2,3-epoxy amines (**445**) with trimethylsilyl trifluoromethanesulfonate (TMSOTf)[264] which opened the epoxide to give the protected hydroxy trifluoromethanesulfonate and then rapidly rearranged on warming to room temperature to give the aziridinium salt (**446**), (**Scheme 94**).




(445) (446)

i. TMSOTf, -78°C, DCM; ii. nucleophile, -78°C to RT; iii. deprotection.

**Scheme 94**

The chemical shifts shown in **Table 20** were calculated using chemical increments[265] for the suspected structures (**443**) and (**444**). However, no value could be found for the phosphonate group and thus an approximation was used for this purpose. The most important chemical shift data for elucidating the structure of the rearranged product, was that of the C-2 and C-3 protons, and their carbon-13 values. Using the chemical shift increments for carbon-13, published by Brown, Floyd and Sainsbury,[265] it appears that the C-2 for (**444**) when corrected will have the lower value as all γ increments for any functionality will have negative values. Whereas for (**443**) the chemical shift increments here will be positive in value as they are β to the centre, thus there will be an increase in the chemical shift for C-2, making the calculated value much higher than the observed value. With respect to the proton n.m.r., the effect on the chemical shift for $CH_2X$ systems, where X=Cl or OH, are very similar. Comparison of the chemical shift of the β-hydroxyphosphonate (**440**) with the

rearranged product show no correlation, thus indicating that they have distinct structures. Further examination of the proton n.m.r. spectra showed that $Me_{Ala}$ appeared at 1.43ppm much higher that for the β-hydroxyphosphonate **(440)** which appears at 1.10ppm. C-2 proton also shows a more complex coupling than would be exhibited for a structure of type **(443)**, here the proton is coupled to three other protons and there is also a $^{1}H$-$^{31}P$ coupling all this shows that path (b) must have been followed to give the γ-chlorophosphonate **(444)**, **Scheme 93**. This is also reinforced by the cosy which shows a strong coupling between the C-4 and C-2 protons, however, no coupling between C-2 and C-3. This may be due to the dihedral angle being close to 90°, giving the coupling value, $J$=0, when the Karplus equation $J$=kcosθ is used, where k is approximately 10.

**Table 20**

| C-H | **Structure** calculated (**443**) (ppm) | | **Structure** calculated (**444**) (ppm) | | **observed** (ppm) | |
|---|---|---|---|---|---|---|
| 1 | 1.83 | 20.9† | 1.8 | 22.2† | 2.3 | 22 |
| 2 | 4.08 | 60.4‡ | 4.00 | 56.7* | 4.4 | 56.7 |
| 3 | 3.13 | 56.4* | 3.33 | 56.4‡ | 3.2 | 62.4 |
| 4 | 0.83 | 22.2 | 1.03 | 20.9 | 1.4 | 22.9 |

As the trifluoromethanesulfonate is a much more reactive species than both the methanesulfonate and *p*-toluenesulfonate, we decided to prepare the more stable *p*-toluenesulfonate derivatives. Kabalka *et al*[266] described the preparation *p*-toluenesulfonates from the corresponding alcohols for the use in subsequent nucleophilic substitution reactions. Secondary alcohols are comparatively less reactive

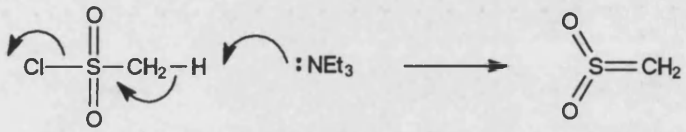
---

† no α increment for phosphonate, values will be much higher

‡ no β increment for phosphonate, values should be higher

* no γ increment for phosphonate, values will be reduced

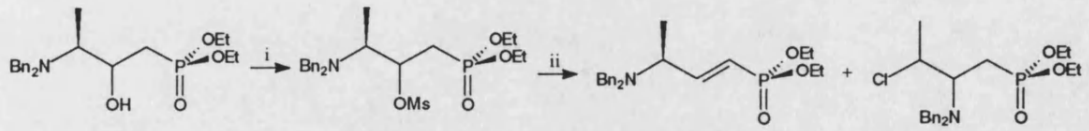
than primary alcohols and require longer reaction times. When we attempted this reaction at room temperature no transformation occurred, even when the reaction was refluxed for 4 hours. Due to our inability to generate the *p*-toluenesulfonate we decided to use the methanesulfonate to activate the alcohol. Crossland and Servis[267] showed that methanesulfonates can be generated quite simply and rapidly even with highly sterically hindered alcohols. They concluded that the mechanistic course of the reaction did not follow the usual nucleophilic addition of the alcohol to the sulfonyl group, but instead addition to a sulfene (**447**) derived from E2 elimination of chlorine from the methanesulfonyl chloride, (**Figure 65**).




(447)

**Figure 65**

In our hands the amino alcohol (**440**) was converted cleanly and rapidly to the corresponding methanesulfonate (**448**) in high yield (90%). Under these conditions no rearrangement occurred and a 60MHz proton n.m.r. spectra showed the characteristic singlet peak for the methyl of the sulfonate at 2.8 ppm. However, the mass spectrum did not show any of the mass ion of 483 a.m.u., but the fragment for the loss of $SO_2Me$ was observed at 406 in (+) FAB. When this was treated with Leu-OMe.HCl the sulfonate (**448**) was converted to a new compound with a higher retention factor by t.l.c., **Scheme 95**.




(436) (448) (449) (444)

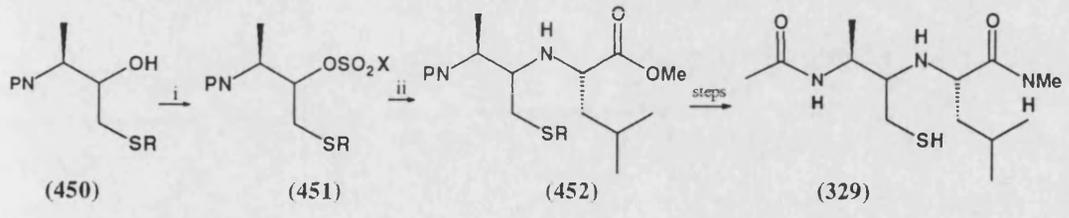
i. Mesyl-Cl, $Et_3N$, DCM, 90%; ii. Leu-OMe.HCl, THF, 1 hr.

**Scheme 95**

Upon analysis of this new compound it was discovered that the chloro compound (**444**) had been formed again. It was interesting to note that the elimination product (**449**), the (*E*)-alkene $J_{trans}$ 17.4 Hz, was also detected as a by-product. As a consequence of these results, this route was abandoned and all efforts were directed towards the other targets.

### 2.6.2 Mercaptomethyl dipeptide (329)

The synthetic strategy for the preparation of the mercaptomethyl dipeptides (**329**) involved ring-opening of the protected amino epoxides, to give the protected hydroxythiols (**450**) (discussed in section **2.5.3**), followed by activation of the hydroxyl moiety to the corresponding sulfonates (**451**), to facilitate formation of a C-N bond with Leu-OMe. In our hands all attempts to furnish these sulfonates (**451**) failed, **Figure 66**.




(450) (451) (452) (329)

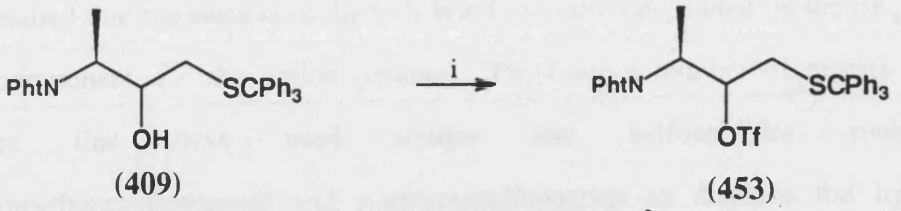
i. a)Triflic anhydride, DCM, -20°C; b) *p*-toluenesulfonyl chloride, pyridine, DCM, RT, 3 days; and c) NaH, *p*-toluenesulfonyl chloride, RT; ii. Leu.OMe.

**Figure 66**

*Attempted preparation of the phthaloylamino aryl thiol triflate* (**453**)

In an attempt to prepare the triflate (**453**) using standard sulfonation techniques, the reaction produced a variety of by-products. N.m.r. spectroscopic studies showed none of them to be the desired product and they were not characterised further. Deprotection of the thiol group was apparent due to the isolation of trityl derivatives. As these conditions were too acidic, and these type of protecting groups were

susceptible to deprotection by strong acids, we opted to use *p*-toluenesulfonate to activate the hydroxyl group, **Figure 67**.



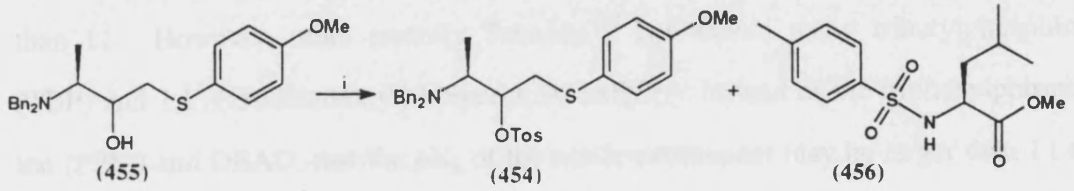

**(409)** **(453)**

i. Triflic anhydride, pyridine, DCM, -20°C.

**Figure 67**

*Attempted preparation of the N,N-dibenzylamino p-methoxyphenylmethylthiol sulfonate* **(454)**

The sulfonation of the *N,N*-dibenzylamino *p*-methoxyphenylmethylthiol alcohol **(455)** did not work using pyridine and *p*-toluenesulfonyl chloride after stirring at room temperature for 3 days. We decided to use NaH to generate the alkoxide anion in the hope that this would react with *p*-toluenesulfonyl chloride to give the desired sulfonate **(454)**. When it was apparent by t.l.c. that some reaction had occurred, we added Leu-OMe. After purification all we isolated was the starting material and the *p*-toluenesulfonyl protected Leu-OMe **(456)**, **Figure 68**.




**(455)** **(454)** **(456)**

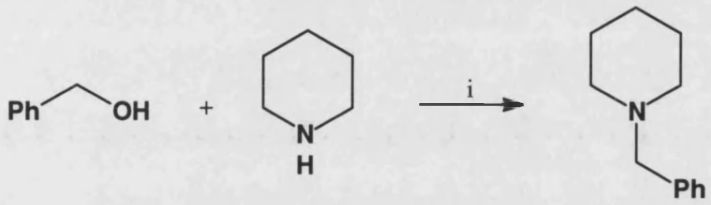
i. NaH, *p*-toluenesulfonyl chloride, RT, Leu-OMe

**Figure 68**

### 2.6.3 Mitsunobu reactions

The Mitsunobu reaction is an exceptionally useful and general method in organic synthesis, whereby a hydroxyl group can be replaced by a vast range of nucleophiles. Extensive reviews by Mitsunobu[268] and Hughes[269] have shown an wide range of

Edwards *et al*[270] described the synthesis of polyamines utilising this useful reaction. They detailed that the success of the C-N bond formation depended on the $pK_a$ of the acidic component, *i.e.* the amide (amine). There are a number of reports in the literature that have used imides and sulfonamides such as trifluoromethanesulfonamide and *p*-toluenesulfonamide to displace the hydroxyl group, but very few detail the use of primary and secondary amines. Sammes and Smith[271] showed that piperidine can be coupled with benzyl alcohol in modest yield (43%), (**Figure 69**).




i. $PPh_3$, DEAD, HCl, THF, DMF, 43%.

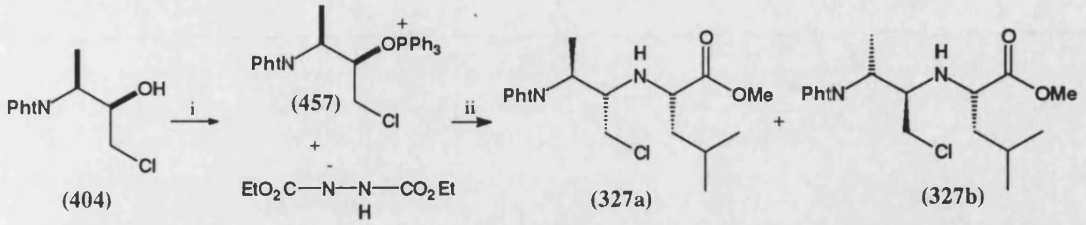
**Figure 69**

In the Mitsunobu reaction it is essential that the *p*Ka of the amide (or amine) is lower than 11. However, more recently Tsunoda[272] has shown using tributylphosphine (TBP) and 1,1'-(azodicarbonyl)dipiperidine, (ADDP), instead of the triphenylphosphine ($PPh_3$) and DEAD, that the $pK_a$ of the acidic component may be larger than 11 to facilitate C-N formation. Edwards *et al*[270] showed that the $pK_a$ of *p*-toluenesulfonamides such as TsNHMe are around 11.7 giving poor yields, but that the $pK_a$'s of TfNHMe type amines were much lower ($pK_a$ ~ 7.5), giving very good yields.

*Preparation of the chloromethyl dipeptide* **(327)**

Our first attempt was a model reaction to identify any possible by-products that may form. We used the racemic *N*-phthaloylamino chloroalcohol **(404)** and the standard

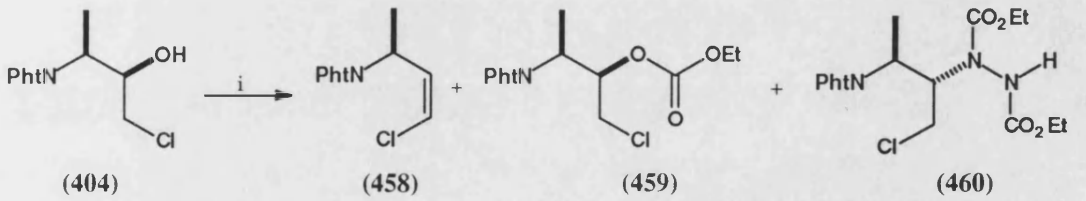
Mitsunobu reagents, $PPh_3$ and DEAD, coupling the activated alcohol **(457)** with Leu-OMe **(396)**, **Scheme 96**.




i. DEAD, $PPh_3$; ii. Leu-OMe.

**Scheme 96**

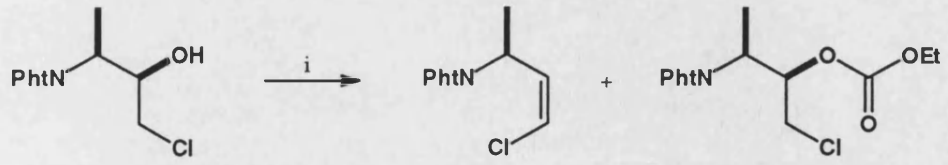
This reaction did not give the expected products. Instead we isolated the racemic (*Z*)-chloroalkene **(458)**, the racemic carbonate **(459)** and the racemic DEAD *N*-alkylation product **(460)**, **Figure 70**.




i. DEAD, $PPh_3$, Leu-OMe

**Figure 70**

NOe studies confirmed the relative stereochemistry of the carbonate as (2*R**,3*S**). The DEAD *N*-alkylation probably occurred because of the acidity of the hydrazine (reduced DEAD) was higher than that of Leu-OMe. Using Boc-Leu-OMe this alkylation did not occur; unfortunately nor did the C-N formation. All that were isolated were the starting materials, (*Z*)-chloroalkene **(458)** and the carbonate **(459)**, **Figure 71**. HPLC of the starting material **(404)** and the carbonate **(459)** using an isocratic run [($CH_3CN$:$H_2O$):$H_2O$ 30:70] gave the carbonate **(459)**, recovered starting material **(404)** and the methyl ketone **(393)**, **Figure 72**.

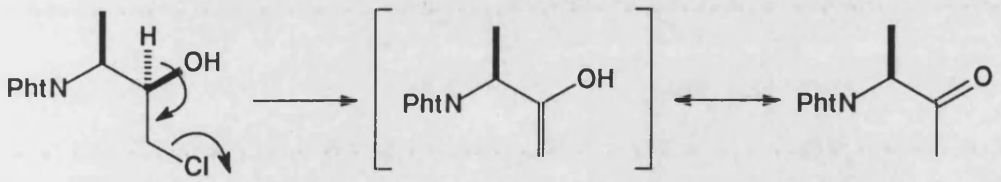



(404) (458) (459)

i. $PPh_3$, DEAD, THF, Boc-Leu-OMe.

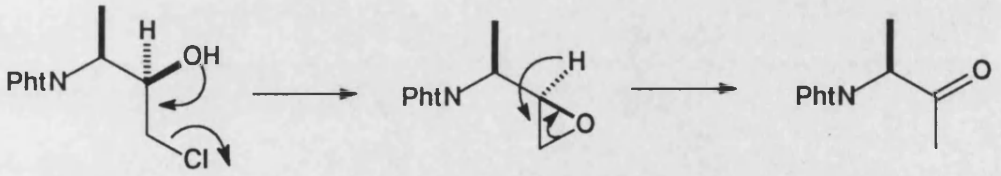
**Figure 71**

The nature of the solvent system, together with silica gel must have resulted in the elimination of the chloro atom and subsequent tautomerisation, to give the methyl ketone (**393**). An alternative and possibly more likely route could involve rearrangement of the epoxide *via* hydride migration. (**Figure 72**)




(404) (393)

or alternatively




(404) (322) (393)

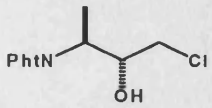
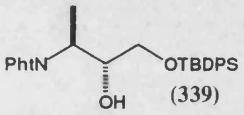
**Figure 72**

The $pK_a$ values of the two leucine esters were both greater than 13 and thus they were not acidic enough to be deprotonated. As we required the Leu-OMe to have a lower $pK_a$, it was decided to make the trifluoroacetyl protected amino ester as removal of the acetyl group was possible using 7% $K_2CO_3$.[273]

The protected amino ester was prepared as detailed in Section **2.2.4**. Its $pK_a$ was experimentally calculated at 10.4.[274] This was just low enough for the Mitsunobu to work. In our hands we could not form the C-N bond between either the racemic

mixtures of the chloroalcohol (**404**) or the protected diol (**339**) and TFA-Leu-OMe (**368**). All that we recovered were the starting materials. **Table 21**

**Table 21**

| alcohol | aminoester | products |
|---|---|---|
|  (**404**) | Leu-OMe | alkene + carbonate<br>DEAD *N*-alkylation |
| (**404**) | Boc-Leu-OMe | alkene + carbonate |
| (**404**) | TFA-Leu-OMe | no reaction |
|  (**339**) | TFA-Leu-OMe | DEAD *N*-alkylation |

*Preparation of the hydroxymethyl dipeptide* (**324**) *and* (**325**)

When the Mitsunobu reaction was attempted on the racemic protected diol (**339**) no coupling occurred with TFA-Leu-OMe (**368**), **Figure 73**. No elimination product was observed, instead 63% of the starting material was recovered together with 9% of the alcohol (**339**) coupled with DEAD to form the complex (**461**) (apparent from $^{1}H$ n.m.r. spectra).


(**339**) (**461**)

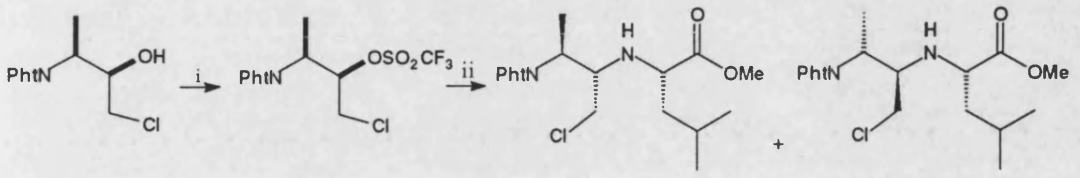
i. $PPh_3$, DEAD, THF, TFA-Leu-OMe.

**Figure 73**

### 2.6.4 Chloromethyl dipeptide

*Preparation of the chloromethyl dipeptides* (**326**) *and* (**462**) *from the trifluoromethanesulfonate* (**463**)

As there were many problems involved with the Mitsunobu we turned our attentions back to the formation of C-N bonds using sulfonates as detailed extensively in section **2.6.1**. The diastereomeric mixture of chloromethyl dipeptides **(327)** and **(462)** were prepared from a racemic mixture of the trifluoromethanesulfonate **(463)**.[247,249,250] The sulfonate **(463)** was generated at -23°C using pyridine and trifluoromethanesulfonate anhydride from the corresponding racemic mixture of the chloroalcohol **(404)**, **Scheme 97**.




**(404)** **(463)** **(327)** **(462)**

i. Trifluoromethanesulfonate anhydride, pyridine, -23°C, 1hr; ii. Leu-OMe.HCl, $Et_3N$.

**Scheme 97**

*Reactions of the chlorotrifluoromethanesulfonates* **(463)** *and* **(464)**

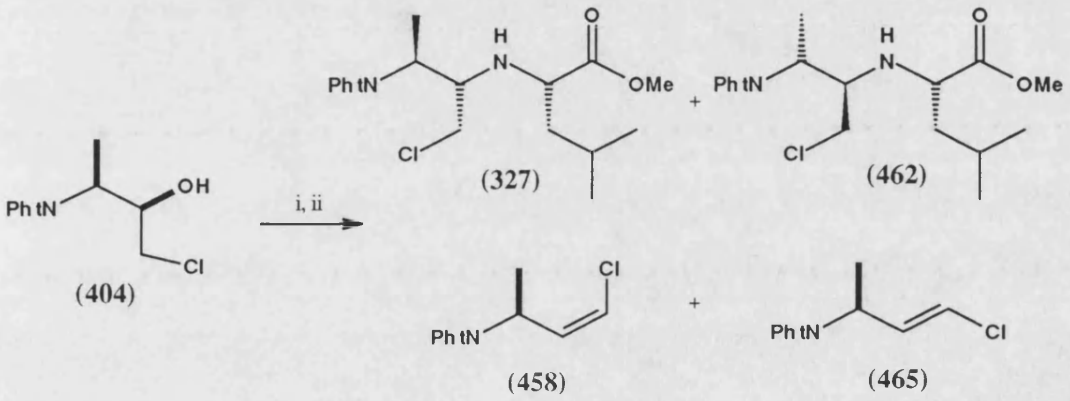
On numerous occasions we managed to isolate the chloromethyl trifluoromethane-sulfonates **(463)** and **(464)** and found them to be rather stable. This was probably due to the β-stabilisation effect of the electronegative chlorine group. They could be easily chromatographed without significant loss of material and were stable to water. Under acidic conditions we found that they were often converted back to the chloroalcohols **(403)** and **(404)**, **Figure 74**.


**(463)** **(404)**

**Figure 74**

Without purification, the racemic sulfonate **(463)** was reacted with Leu-OMe **(396)**, generated *in situ* by treatment of Leu-OMe.HCl and triethylamine in DCM, **Figure 75**.

The coupling reaction did not go cleanly as there was a considerable amount of the elimination products formed. The chloromethyl dipeptide diastereoisomers (2*S*,3*S*,2'*S*) (**327**) and (2*R*,3*R*,2'*S*) (**462**) [2:1 ratio (observed by $^1$H n.m.r. spectra)] were isolated in 34% yield. Also isolated were the (*Z*)- and (*E*)-chloroalkene (**458:465**, 95:5) (12% yield), produced *via* elimination of a proton *alpha* to the chloro group. $^1$H n.m.r. spectra showed the alkene to be predominately of the *cis* geometry ($J_{CHMe\text{-}H}$ 7.3 Hz and $J_{cis}$ 8.0 Hz).



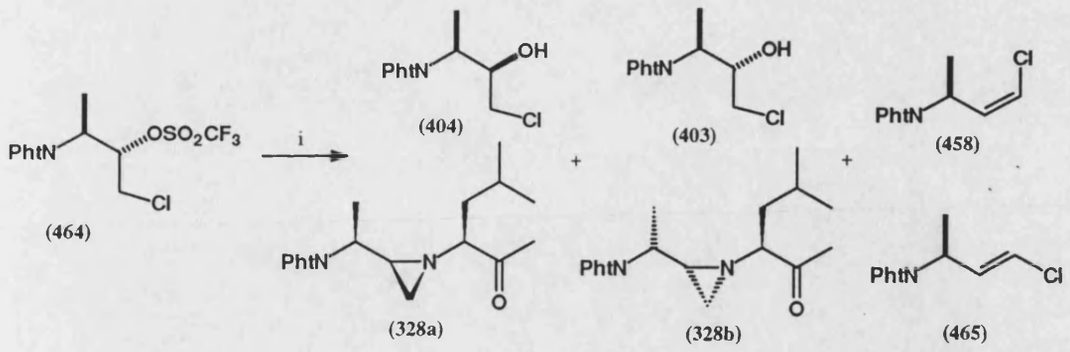

i. Trifluoromethanesulfonate anhydride, pyridine, DCM, -23°C, 1hr; ii. Leu-OMe.HCl, Et3N, 2 hrs.

**Figure 75**

Unfortunately we found that the diastereoisomers (**327**) and (**462**) could not be separated by flash chromatography.

When this reaction was repeated we used an excess of base and left the reaction mixture stirring for 2 weeks. None of the chloromethyl dipeptide (**327**) was isolated. Instead the chloroalcohols (**403:404**, ~1:1, 58%) were isolated, together with the chloroalkene (**458**) (5%) and the aziridine (**328**) (8%) $R_f$ 0.14 [EtOAc:petrol (30:70)], **Figure 76**.

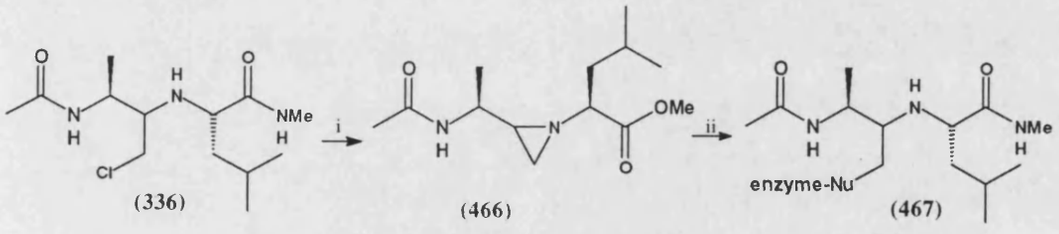
These conditions must have allowed the formation of the chlorodipeptides (**327**) and (**462**) but due to the excess amount of base and the long reaction time, elimination to the chloroalkenes or intramolecularly cyclisation to aziridine (**328**) occurred.




i. Leu-OMe.HCl, excess $Et_3N$, THF, 2 weeks, RT.

**Figure 76**

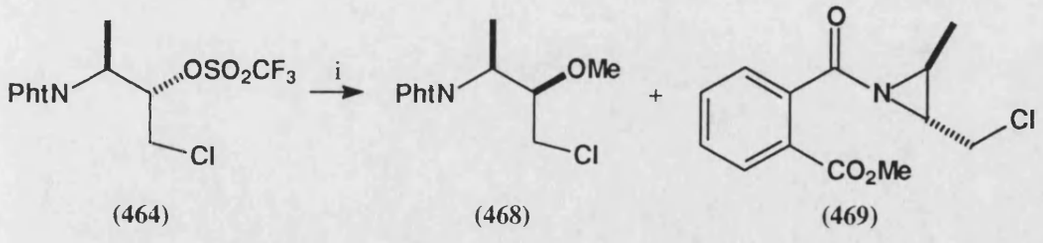
The aziridines (**328**) and the chloromethyl dipeptide (**327**) are members of two new families of dipeptide mimetics, which have the possibility of being either irreversible (**Scheme 98**) or reversible inhibitors. The chloromethyl dipeptide (**327**) has the potential of being converted to the corresponding aziridine (**328**) *via* enzyme degradation, (**Scheme 98**).




i. enzyme-base; ii. enzyme-Nu.

**Scheme 98**

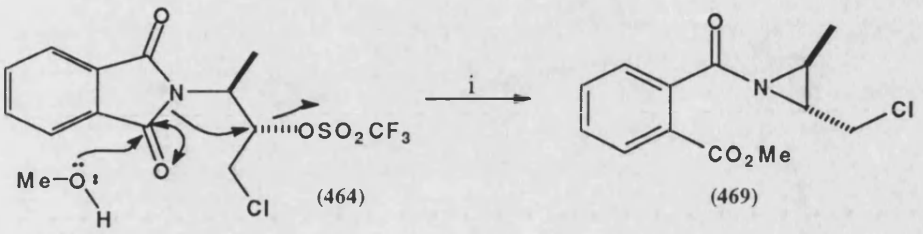
The aziridines have been shown to be very reactive species (section **2.5.1**, **Scheme 82**) and as a consequence may react with enzymes forming the complex (**467**).




i. $K_2CO_3$, MeOH, Leu-OMe.HCl, 1 day.

**Figure 77**

We tried to reduce the amount of elimination products by changing the base and the solvent. The use of $K_2CO_3$ in MeOH, resulted in the formation of the corresponding methoxychloro compound **(468)** (6%) and the aziridine **(469)** (69%). Only a very small amount of the chloromethyl dipeptide was detected by n.m.r. spectroscopy (<1%), **Figure 77**. The aziridine **(469)** was most probably formed *via* the mechanism shown below, **Figure 78**.

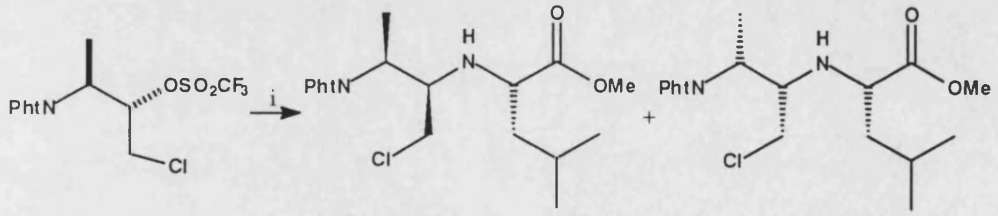



(464) (469)

i. MeOH, $K_2CO_3$, DCM.

**Figure 78**

However, when $K_2CO_3$ in DCM was used we successfully managed to make the chloromethyl dipeptide **(326)** without elimination occurring, although isolated in low yield (30%). Similarly, the coupling reaction was achieved using the hindered base DIPEA in DCM, **Figure 79**.




**(464)** **(326)** **(470)**

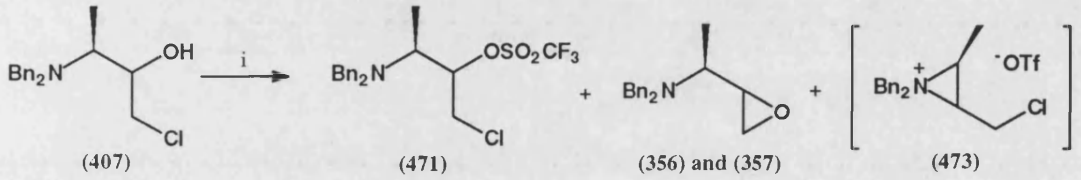
i. $K_2CO_3$, DCM, Leu-OMe.HCl, 2 hrs.

**Figure 79**

A new family of reduced dipeptides have therefore been established. These are related to the Szelke reduced amides described in the Introduction (Section **1.4**). They are potentially more conformationally constrained because of the chloromethyl substituent, and might also act as reversible or irreversible enzyme inhibitors (the later as a consequence of the aziridinium intermediates, **Scheme 98**).

*Attempted preparation of the dibenzylamino chloro trifluoromethanesulfonate* **(471)**

We attempted to convert *N,N*-dibenzylamino chloroalcohol **(407)**, which was made in an analogous way to the *N*-phthaloylamino chloroalcohol **(404)**, to the chloromethyl dipeptide **(472)** using the same methodology just discussed. When we attempted to form the trifluoromethanesulfonate **(471)** using standard conditions it was discovered that many by-products occurred, including regeneration of the epoxides **(356)** and **(357)**. We believe, even though we did not isolate these products, that the desired sulfonate **(471)** was formed together with the aziridinium salt **(473)** and other products, **Figure 80**.




(407) (471) (356) and (357) (473)

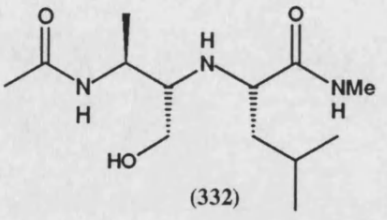
i. Trifluoromethanesulfonate anhydride, pyridine, DCM, -23°C, 1hr.

**Figure 80**

With this disappointing result, we abandoned this particular approach.

### 2.6.5 Hydroxymethyl dipeptide

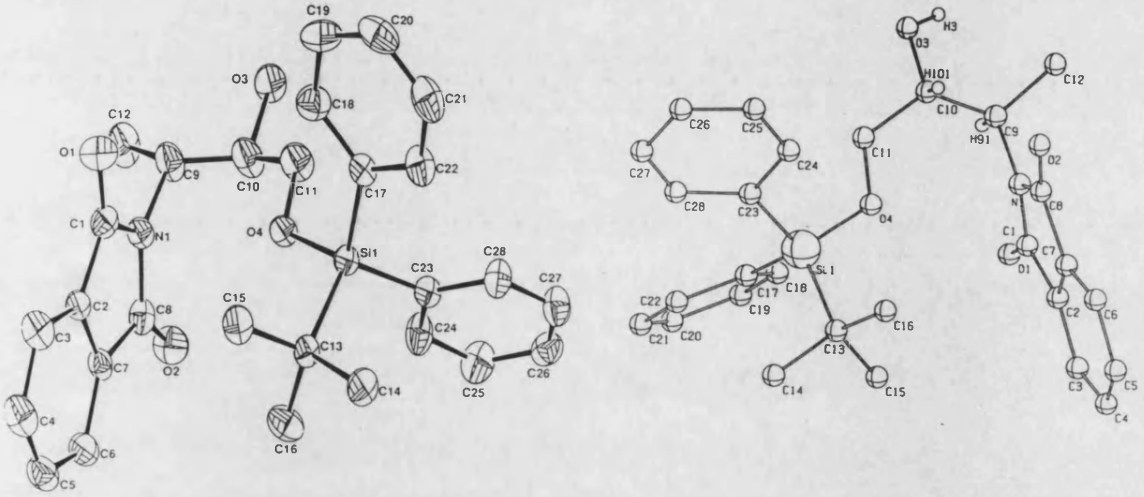
A further new family of potential enzyme inhibitors which were targeted had the general structure **(332)**. Diastereoselective routes were therefore required to prepare this new pseudopeptide **(332)**.




(332)

Due to the problems encountered with the direct formation of the hydroxymethyl dipeptide from the addition of Leu-OMe to the *N*-phthaloylamino epoxide **(322)** and the failed attempts at the coupling using triphenyl phosphine and DEAD, a different approach was adopted. This involved the protection of the primary alcohol of the diols

(**337:338**) and then activation of the secondary alcohol, hopefully facilitating the formation of a C-N bond with Leu-OMe. The primary alcohol in the mixture of diols was protected by *tert*-butyldiphenylsilane[275,276] (TBDPS) to give alcohols (**339**) and (**474**) in 82-98% yield following a modified procedure of Corey *et al.*[277] The reaction was complete in 30 minutes when carried out in DCM at 30°C. The diastereoisomers could easily be separated by chromatography and were crystalline enough for X-ray crystallography, **Figure 81**.



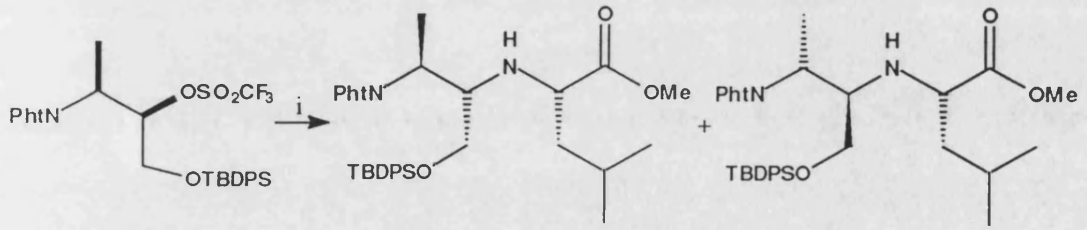

X-ray structure of the protected diol (**474**) with the stereochemical configuration 2*R*,3*R*

**Figure 81**

The stereochemistry of all the following products were assigned from this single X-ray. The protected diol (**474**) from which the X-ray was obtained, was prepared from the racemic aldehyde (**350**). In summary, all the following material was racemic, olefination and then epoxidation (*m*-CPBA) gave the *threo* isomer (namely 2*R*,3*S*) as the major isomer (**322**) as described by Luly *et al.*[143h] Acid catalysed ring-opening gave the diols (**337:338**) as a mixture (91:9). These were inseparable, and their stereochemistry unknown due to the addition of water *via* a mixture of $S_N1$ and $S_N2$ mechanism. However, hydroxyl protection allowed for their separation and as they

were crystalline, X-ray crystallography of the minor isomer showed it to be a racemic mixture of the isomer (2*R**,3*R**) (**474**) in a 1:1 ratio, the X-ray was of the (2*R*,3*R*) isomer.

A model synthesis was performed using the racemic diols (**337**) (2*R**,3*S**) and (**338**) (2*S**,3*S**) to establish the best way of producing the hydroxymethyl dipeptide (**Figure 82**). The protected diols (**339**) (2*R**,3*S**) and (**474**) (2*S**,3*S**) were prepared, separated and the major isomer (**339**) was then successfully converted to the corresponding trifluoromethanesulfonate (**475**) following the procedure outlined by Effenburger *et al.*[250]




(475) (476) (477)

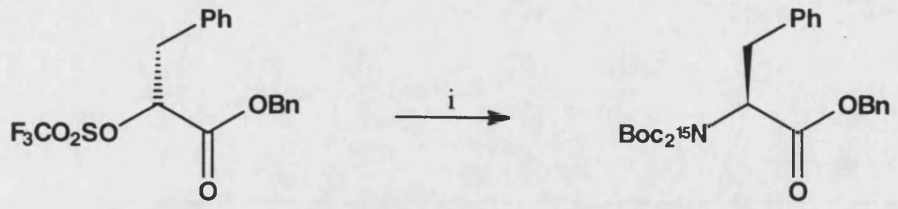
i. Leu-OMe.HCl, base, DCM or THF, RT or reflux.

**Figure 82**

The sulfonate (**475**) was typically isolated in good yield (73-89%). Degerbeck *et al*[253] showed that $^{15}NHBoc_2$ when treated with *n*-BuLi at lowered temperature converted α-trifluoromethanesulfonate ester (**478**) to the corresponding α-amino ester (**479**) in good yield. The additions went smoothly, with Walden inversion and no loss of enantiomeric purity. † **Figure 83**

---

† We recognise the possibility that a double inversion my occur involving displacement of the triflate group by one of the phthaloyl carbonyl groups. Thus, producing the isomer of stereochemical configuration (2*R*,3*S*,2'*S*) which could would correlate to the isomer produced via ring-opening of the epoxides (**322**) and (**323**).

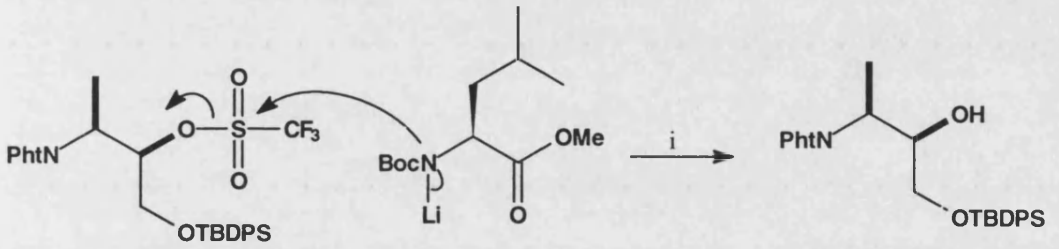



(478) (479)

i. $^{15}NHBoc_2$, *n*-BuLi, THF, -78 to -30°C, 16 hrs, 92%.

**Figure 83**

In our hands, there was no coupling, instead the trifluoromethanesulfonate was removed to give the protected diol **(339)**, **Figure 84**.

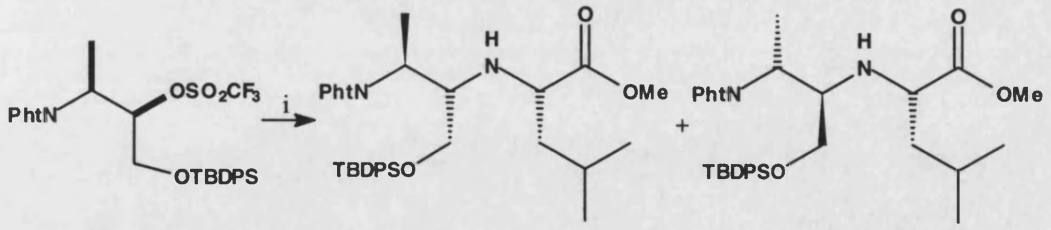



(475) (339)

i. THF, -78°C, 1 hr, 0°C, 2 hrs, RT, 14 hrs.

**Figure 84**

As these conditions did not give us the dipeptide, we decided to displace the trifluoromethanesulfonate at room temperature as Effenberger *et al*[250] had reported. At our first attempt, 40% of the trifluoromethanesulfonate **(475)** was coupled in 3 days at room temperature with half the starting material recovered, **Figure 85.**

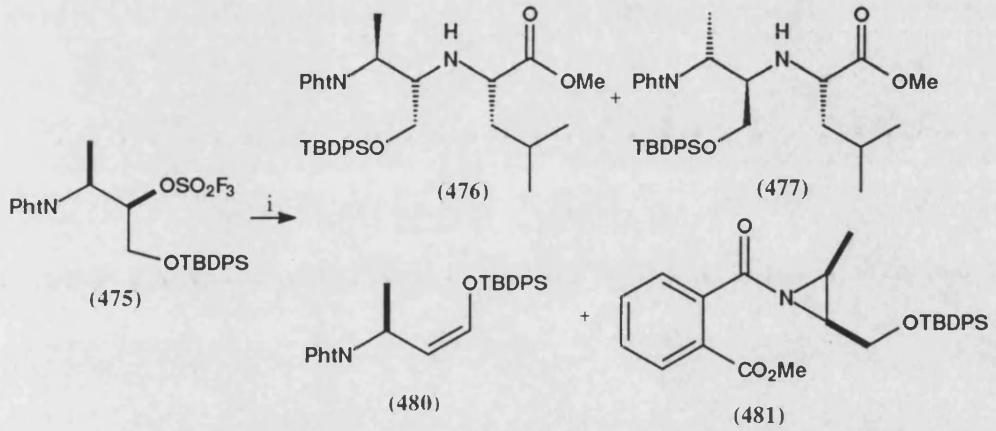



(475) (476) (477)

i. Leu-OMe.HCl, $Et_3N$, DCM, RT, 3 days.

**Figure 85**

IR showed the NH at 3388cm$^{-1}$ and the ester at 1735cm$^{-1}$ and the $M^+$+1 was at 601 a.m.u. The proton n.m.r. was comparable with that of the hydroxymethyl dipeptide prepared from the epoxides (**322**) and (**323**), the $Me_{Ala}$ was seen at 1.23ppm. We mistakenly decided to use MeOH as the solvent in another attempt to increase the yield of the coupling step. This resulted in aziridine (**481**) as the major product (see **Figure 86**). However, small amounts of the desired product (**476**) and (**477**), the silylether (**480**) and the aziridine (**481**) were also observed by n.m.r. spectroscopy.




i. Leu-OMe.HCl, MeOH, $K_2CO_3$, RT, 1 day.

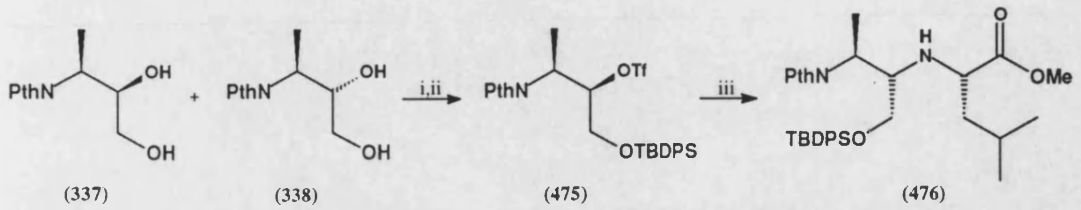
**Figure 86**

Other variations in solvent, base, and temperature are shown in **Table 22**.

**Table 22**

| Solvent | Base | Eqiv.s | temp. (°C) | Recovered s.material | Yield of product | Alkene |
|---|---|---|---|---|---|---|
| DCM | TEA | 1 | 0 to RT | 50% | 40% | 0% |
| THF | DIPEA | 2 | reflux 2hrs | 50% | 31% | |
| DCM | DIPEA | 2 | reflux 12hrs | 19% | **56%** | 2.5% |
| DCM | DIPEA | 1 | reflux 8 hrs | 0% | 36% | 31% |

As this route proved successful for the racemic compounds, the enatiomerically pure dipeptide (**476**) was prepared in an analogous manner, **Figure 87**, in an overall yield of 45% from the mixture of diols (**337**) and (**338**), (91:9).




(337) (338) (475) (476)

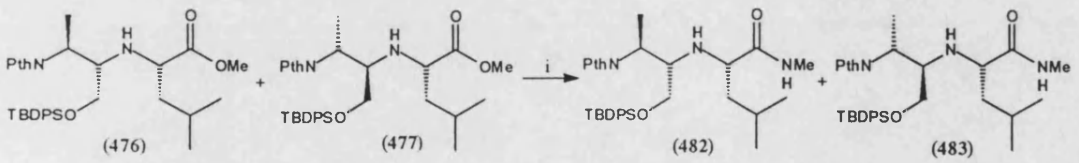
i. TBDPS-Cl, DCM, 30°C, ½hr, 98%; separate by chromatography; ii. $Tf_2O$, pyridine, DCM, -23°C, 89%; iii. Leu-OMe, DIPEA, DCM, reflux, 12hrs, 56%.

**Figure 87**

## 2.7 Deprotection and Amidation

### 2.7.1 Amidation

Genin *et al*[278] had shown that esters could be clearly converted to their corresponding amides, using methylamine in MeOH. The mixture of the silyl protected hydroxymethyl dipeptide isosteres (**476**) and (**477**) were converted to the corresponding amides (**482**) and (**483**) using excess methylamine, in poor yield, typically about 20%, **Figure 88**. The n.m.r. spectra showed the presence of the NH*Me* at roughly 2.6ppm, two peaks were observed, showing that two rotamers were present. When this was attempted on the single isomer (**476**) the corresponding amide (**482**) was isolated in low yield (22%). IR of this compound showed that the NH peak was observed at 3407cm$^{-1}$ and the methylamide at 1658cm$^{-1}$, accurate mass spectroscopy showed that the $M^{+}$+1, was at 600.3254 a.m.u. for the expected 600.3257. Proton n.m.r. showed the presence of the N*Me* as two rotamers at 2.62 and 2.64ppm, the $Me_{Ala}$ was observed at 1.33ppm all other peaks were comparable to those observed for the precursor (**476**), see **Table 24.**




i. $MeNH_2$, MeOH, RT, 4 hrs, 22%.

**Figure 88**

### 2.7.2 Deprotection

Silyl protecting groups can be removed in a manner of ways. Tetrabutylammonium fluoride has been used extensively to this end. When we attempted this on the mixture of hydroxymethyl dipeptides (**476**) and (**477**), the corresponding alcohols (**325**) were isolated in good yield (63%). Mass spectroscopy showed that the mass ion was at 363

a.m.u. in FAB (+) spectra. The proton n.m.r. spectra showed that the ester group was still intact, *i.e.* no lactonisation had occurred. Comparison of the proton n.m.r. data for the preparation of the hydroxymethyl dipeptide **(325)** from the protected diol **(474)**, with that of the epoxide opened hydroxymethyl dipeptide **(324)**, **Table 23**, shows clearly seen that they are not comparable. This indicates that each dipeptide is stereochemically unique.

**Table 23**

| Proton number | Isomer (**325a**) (*2S,3S,2'S*) $\delta_H$ ppm | Isomer (**324b**) (*2R,3S,2'S*) $\delta_H$ ppm | Isomer (**324a**) (*2S,3R,2'S*) $\delta_H$ ppm |
|---|---|---|---|
| 3 | 4.67 | 4.89 | 4.97 |
| 2 | 4.00-4.10 | 3.89-3.95 | 4.09-4.18 |
| 1 | 3.60-3.70 | 3.49, 3.50 | 3.40-3.53 |
| 2' | 5.10 | 5.07 | 5.09 |
| $Me_{Ala}$ | 1.41 | 1.52 | 1.54 |
| OMe | 3.78 | 3.74 | 3.77 |
| $Me_{Leu}$ | 1.03 | 1.03 | 1.03 |
| $Me_{Leu}$ | 0.91 | 0.91 | 0.91 |

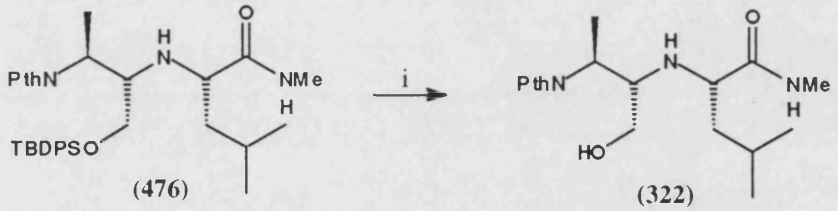
*Preparation of the hydroxymethyl dipeptide from the corresponding protected precursor* **(482)**

As has been discussed earlier, the hydroxymethyl dipeptides **(324)** and **(325)** can be prepared directly *via* ring-opening off the epoxides **(322)** and **(323)** by the protected amino ester (Leu-OMe). The alternative route which is discussed in this section, can produce both the (*2S,3S,2'S*) and (*2R,3S,2'S*) configurations without contamination by minor impurities, such as the diols **(337)** and **(338)**. The protected hydroxyl groups were successfully deprotected to give the desired dipeptides **(322)** in good yield (67%). IR and mass spectroscopy were consistent with the authentic product, however, the proton n.m.r. differed slightly as they were different diastereoisomers. **Tables 23** and **24**.

**Table 24** shows the comparative n.m.r. data for the backbone of selected dipeptides.

**Table 24**

| Proton number | Isomer (**322**) (*2S,3S,2'S*) $\delta_H$ ppm | Isomer (**482**) (*2S,3S,2'S*) $\delta_H$ ppm | Isomer (**476**) (*2S,3S,2'S*) $\delta_H$ ppm | Isomer (**324b**) (*2R,3S,2'S*) $\delta_H$ ppm |
|---|---|---|---|---|
| 3 | 4.94 | 5.17 | 5.20 | 4.89 |
| 2 | 4.14 | 4.17-4.26 | 4.01-4.17 | 3.89-3.95 |
| 1 | 3.51<br>3.63 | 3.63<br>3.83 | 3.50-3.60<br>3.84 | 3.49<br>3.50 |
| 2' | 4.83 | 5.05 | 5.02-5.12 | 5.07 |
| 3'and 4' | 1.58-1.95 | 1.65-1.83 | 1.68-1.98 | 1.70-1.99 |
| $Me_{Ala}$ | 1.44 | 1.33 | 1.23 | 1.52 |
| $Me_{Leu}$<br>$Me_{Leu}$ | 0.89<br>0.81 | 0.96<br>0.87 | 1.03<br>0.91 | 1.03<br>0.91 |



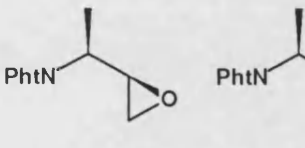

i. TBAF, THF, RT, 1 hour, 67%.

**Figure 89**

## 2.8 Summary

The synthesis of (2*R*,3*S*,2'*S*)-hydroxymethyl dipeptide (**324b**) has been described, from the readily available amino acid L-alanine, *via* the homochiral epoxides (**322**) and (**323**). Improved synthesis of the later has been developed, with the problem of the low yielding Wittig reaction being resolved and the epoxides being produced enantiomerically pure, suitable as precursors to a number of novel analogues (**337**), (**404**), and (**409**). The diols (**337**) and (**338**) were successfully converted to the single isomer hydroxymethyl dipeptide mimetic (**332**). Also described is the synthesis of the novel hydroxyethylamine (**398**) and the new family of transition-state analogues, aziridine (**328**). The preparation of another new family of transition-state analogues, the chloromethyl dipeptide mimetics of general structure (**326**) is also described.

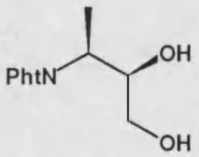
The use of the *N*,*N*-dibenzyl protecting group gave the β-hydroxyphosphonate (**440**) and the Gordon type hydroxyethylamine (**402**).



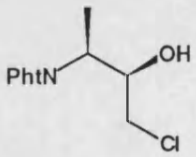

(322)



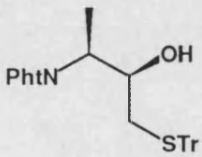

(323)



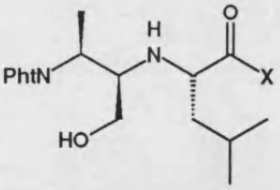

(337)



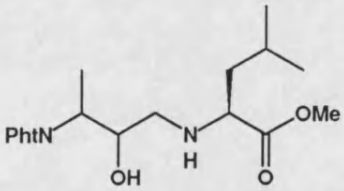

(404)



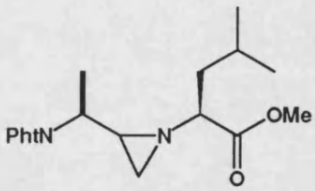

(409)



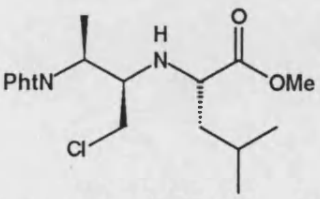

X=OMe (324), NHMe (332)



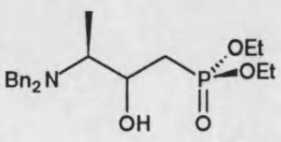

(398)



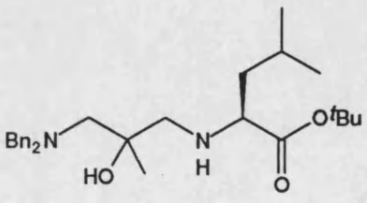

(328)




(326)




(440)




(402)

# CHAPTER THREE

## 3.0 Molecular Modelling

### 3.1 Generation of the structures

The pseudotripeptide structures **(485)** and **(486)** were built using the molecular modelling suite INSIGHT.[279] These were initially based upon the tripeptide Ac-Ala-Leu-NHMe **(487)**. The carbonyl bond between Ala and Leu was replaced with the transition-state mimic unit $CH_2OH$. For the purpose of human physiological studies the pseudotripeptides were protonated, carrying a charge of +1. The charges assigned by the program INSIGHT, gave an overall charge of +0.22. Using single point calculations the charges on each atom were amended so that the overall charge would be *ca.* +1. The program GAUSSIAN 92[280] was used and the values around the quaternary nitrogen were derived so that the charge requirement could be reached. Similar values and not the exact values were used, **Figure 90**.

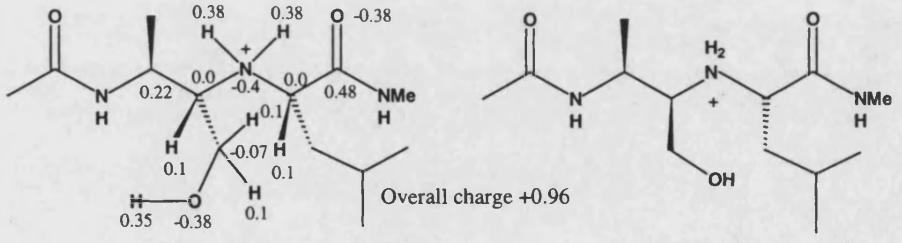



**Figure 90**

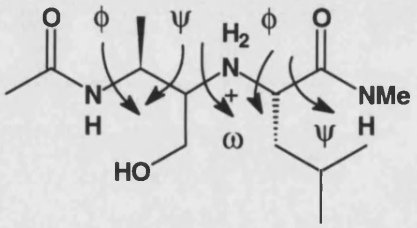
As it is a popular belief that many peptides bind to enzymes in a β-turn conformation, we decided to calculate β-turns for these pseudotripeptides to see if they could be implicated here. With the results obtained from these calculations we wanted to find the lowest energy conformers. This was achieved by generating ϕ, ψ maps which gave us the low energy regions corresponding to distinct confomers. From this data it was possible to find the lowest energy conformer suitable for docking into the active site of collagenase, with the view to finding a relative binding energy for the isomers **(485)** and **(486)**.

## 3.2 Calculating β-turn energies

The generated pseudotripeptides **(485)** and **(486)** had their torsion angles set to the required values for the specific β-turn type (see **Table 25**) and these conformations were minimised using *Steepest descents* for 100 iterations or until the maximum derivative was less the 5 Kcal/mol. Followed by a modified *Newton-Raphson* for 1000 iterations or until the maximum derivative was less the 0.05 Kcal/mol., forcing the ϕ, ψ angles. The conformations for the extended structures were choosen so that they corresponded to the energy minimas on the ϕ, ψ maps. All these calculations were performed using the DISCOVER[279] molecular modelling package.

**Table 25**

| | Ala | | Leu | |
|---|---|---|---|---|
| β-turn | ϕ | ψ | ϕ | ψ |
| I | -60 | -30 | 90 | 0 |
| II | -60 | 120 | 80 | 0 |
| III | -60 | -30 | -60 | -30 |




*Native dipeptides*

The minimised conformations of the three standard β-turns and the extended conformations of the Ac-Ala-Leu-NHMe **(487)** are shown in **Table 26**. Types I and III are very similar and only differ in the ϕ and ψ of the Leu residue (by ≈30°) and are the most stable β-turn conformers. Type II is less stable since the Leu side chain is in close proximity to the Ala carbonyl. These results are in agreement with those reported for the native dipeptides -Ala-Ala- by Dauber-Osguthorpe *et al.*[281]

**Table 26**

| β-turn type | Ala | | | Leu | | Kcal/mol | |
|---|---|---|---|---|---|---|---|
| | ϕ | ψ | ω | ϕ | ψ | energy | rel. energy |
| I | -58.9 | -29.3 | 170.0 | -87.1 | -1.5 | 1.9 | 6.1 |
| II | -63.7 | 115.5 | -168.5 | 74.7 | -0.5 | 5.1 | 9.3 |
| III | -58.4 | -28.7 | 167.0 | -64.8 | -28.3 | 1.8 | 6.0 |
| extended | -83.2 | 85.4 | -173.3 | -89.7 | 84.5 | -4.2 | 0 |

*Hydroxymethyl methylamine dipeptides*

The β-turns and the extended conformations were all relatively less energetically favourable than those for the corresponding native dipeptides, due to the coulombic interactions with the charged nitrogen species and steric clashes between the Leu aliphatic side chain and the acetyl group on the N-terminal. **Table 27**, shows that the β-turn types I and III are again the more stable, and type II was again less stable due to $CH_2OH$ moiety and the Leu aliphatic side chain van der Waals interactions. For both the isomers the β-turn type I exhibits steric interactions between the $CH_2OH$ moiety and the Ala and Leu aliphatic side groups. For type III β-turns there is a clash between the acetyl carbonyl and the Leu residue.

These results are similar to those reported by Dauber-Osguthorpe *et al*[281] for the reduced pseudotripeptide Ac-Ala-ψ[$CH_2NH$]-Ala-NHMe, where the β-turn type II conformation has the least favoured geometry and the β-turn types I and III are of comparable energy. This suggests that the relative stabilities are due to the $sp^3$ character of the modified carbonyl.

**Table 27**

| β-turn type | Ala | | | Leu | | Kcal/mol | |
|---|---|---|---|---|---|---|---|
| | ϕ | ψ | ω | ϕ | ψ | energy | rel. energy |
| isomer (2*S*,3*S*,2'*S*) **(485)** | | | | | | | |
| I | -60.6 | -32.6 | 163.4 | -88.9 | -0.1 | 43.0 | 15.3 |
| II | -61.4 | 113.3 | -170.9 | 80.2 | 0.7 | 51.1 | 23.4 |
| III | -59.7 | -31.5 | 164.0 | -59.7 | -30.5 | 37.7 | 10.0 |
| extended | -78.2 | 70.4 | 127.1 | -73.4 | 152.8 | 27.7 | 0 |
| isomer (2*R*,3*S*,2'*S*) **(486)** | | | | | | | |
| I | -58.1 | -28.3 | 173.7 | -90.5 | -0.9 | 42.4 | 15.1 |
| II | -61.9 | 120.5 | -163.4 | 76.7 | -0.5 | 53.7 | 26.4 |
| III | -58.5 | -28.7 | 165.0 | -63.1 | -31.3 | 43.4 | 16.1 |
| extended | -84.4 | 61.6 | -129.2 | -81.7 | 136.5 | 27.3 | 0 |

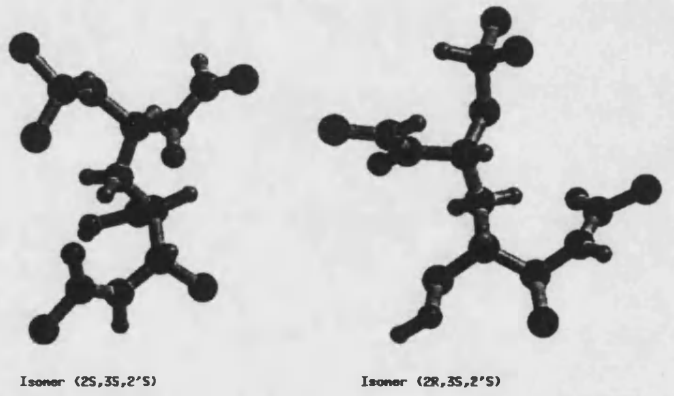



**Figure 91**

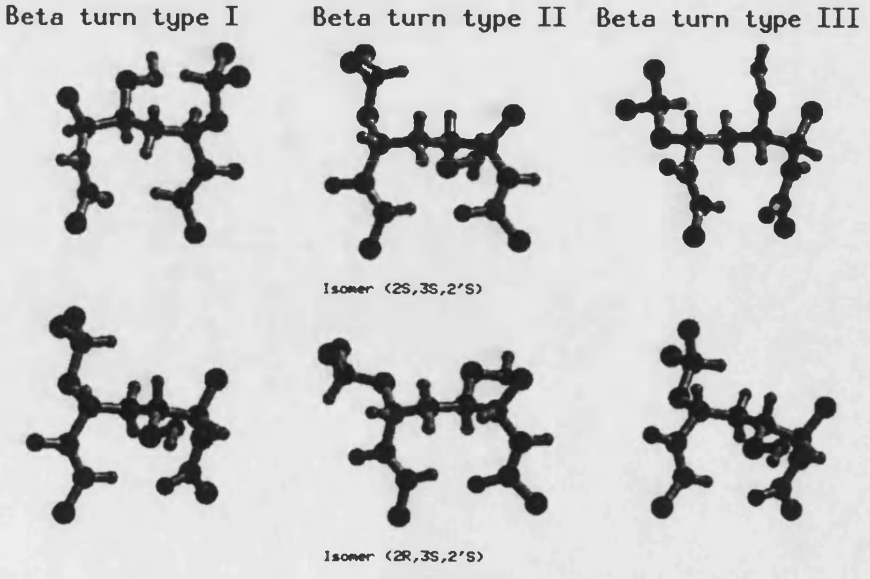



**Figure 92**

## 3.3 ϕ, ψ Maps

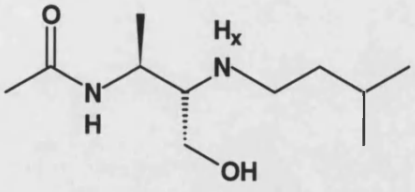
### 3.3.1 Generation of the ϕ, ψ maps

The pseudotripeptides **(484)** and **(485)** were minimised, to reduce any unfavourable clashes *etc.*, using *Steepest descents* followed by a modified *Newton-Raphson*. The resultant structures was then used to generate ϕ, ψ maps. These give an insight into the conformational freedom available to the molecule. This process involves setting the ϕ and ψ angles to pre-set values and forcing these to remain constant whilst the rest of the molecule is then minimised. This follows the methodology described by Dauber-Osguthorpe *et al.*[281]

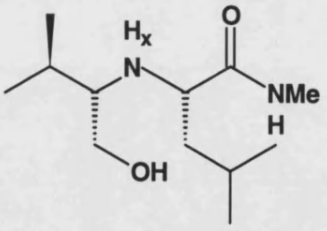
### 3.3.2 Methodology

The strategy was to explore the complete conformational space available to the reduced peptide mimetic and use these to examine the free energy of binding with collagenase. ϕ, ψ Maps were performed upon the charged and the uncharged transition-state mimetic **(484)** and **(485)** in an attempt to elucidate the preferred conformations.

The molecule was spilt into two portions, the Ala residue **(488)** and the Leu residue **(489)**, **Figure 93**.




X=1 or 2 **(488)**




**(489)**

**Figure 93**

*Charged species*

The $\phi$, $\psi$ energy map of the Ala residue (**488**) indicates two main low energy regions. One to the bottom right of the map at $\phi$, 30° to 80°, $\psi$, -50° to -80° and the lowest energy conformation is seen in the top left region of the map at $\phi$, -60° to -100°, $\psi$, 30° to 80°. These are low in energy due to H-bonding between the H-$N^+$ and the acetyl carbonyl (distance ~2.0Å). The rotational energy barrier between these two conformationally favoured regions is 10-12 Kcal/mol. The lowest region is represented by a **1** and the next lowest a **2** *etc.*

The $\phi$, $\psi$ energy map of the Leu residue (**489**) indicates two distinct low energy regions on the left hand side of the map at $\phi$, -80° to -100°, $\psi$, -80° to -120° and the lowest energy conformation is seen in the top left region of the map at $\phi$, -60° to -120°, $\psi$, 120° to 160°. The rotational energy barrier between the two conformationally favoured regions is 22-24 Kcal/mol. Again the lowest region is represented by an **1** and the next lowest a **2** *etc.*

*Uncharged species*

The $\phi$, $\psi$ energy map of the Ala residue (**488**) uncharged unit is similar to the charged, but here the lowest conformation no longer exists at $\phi$, 60°, $\psi$, -70° instead a new lowest conformation is observed at $\phi$, -50° to -80°, $\psi$, -70° to -120°, this is due to the $CH_2OH$ proton H-bonding to the acetyl carbonyl. The other low energy conformer is still seen $\phi$, -60° to -100°, $\psi$, 30° to 80° where the Ala methyl lies gauche to the $CH_2OH$ moiety and is anti-periplanar to the backbone. The rotational energy barrier between these two conformationally favoured regions is now 18 Kcal/mol passing through a low energy region on the map at $\phi$, 50° to 70°, $\psi$, -100° to -160°.

The $\phi$, $\psi$ energy map of the Leu residue (**489**) uncharged is almost unchanged both

the distinct low energy regions have expanded and the lowest region now lies to the bottom left hand side of the map at ϕ, -50° to -110°, ψ, -30° to -100° and the other low energy region at the top left hand side region of the map at ϕ, -40° to -150°, ψ, 90° to 150°. The rotational energy barrier between the two conformationally favoured regions is now 6 Kcal/mol.

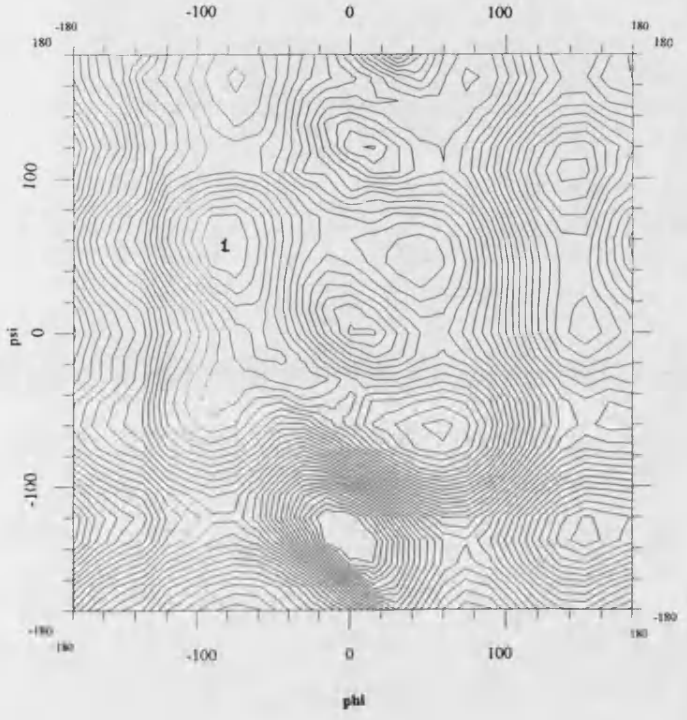



**Figure 94**. ϕ,ψ map of the *charged* Ala residue **(488)**, the global minima is represented by **1**, contours at 2 kcal/mol.

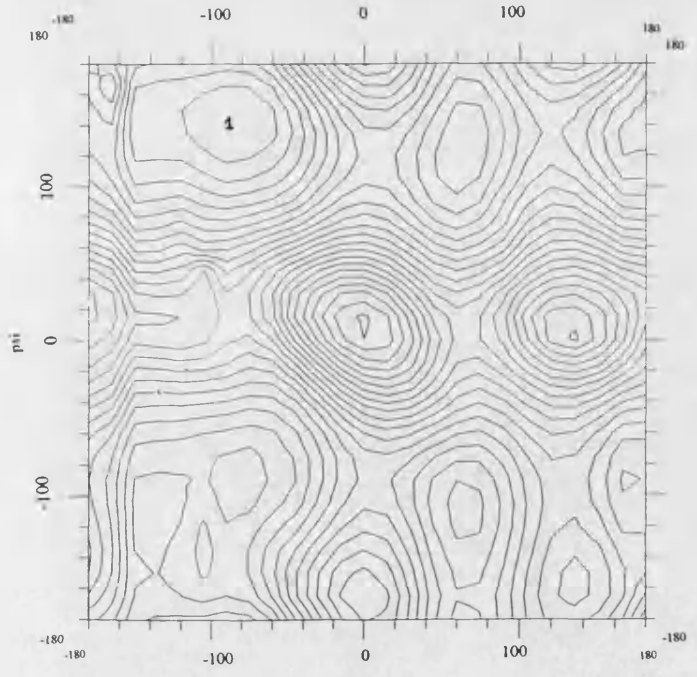



**Figure 95**. ϕ,ψ map of the *charged* Leu residue **(489)**, the global minima is represented by **1**, contours at 2 kcal/mol.

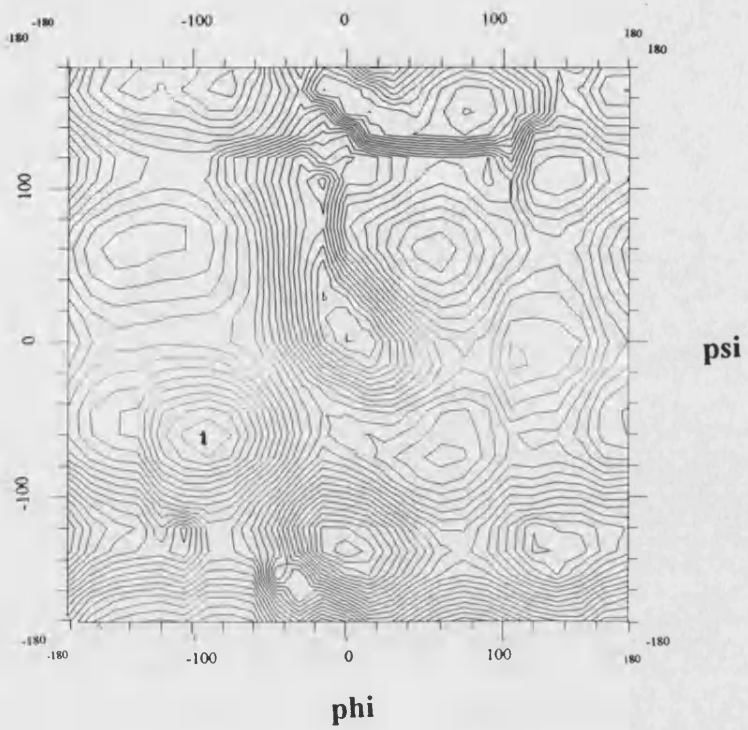



**Figure 96**. ϕ,ψ map of the *uncharged* Ala residue **(488)**, the global minima is represented by **1**, contours at 2 kcal/mol.

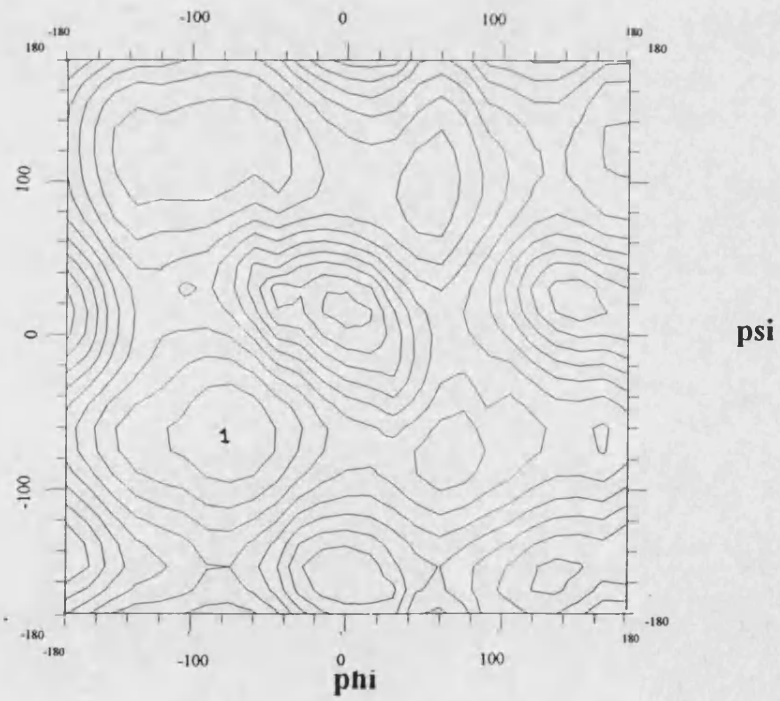



**Figure 97**. ϕ,ψ map of the *uncharged* Leu residue **(489)**, the global minima is represented by **1**, contours at 2 kcal/mol.

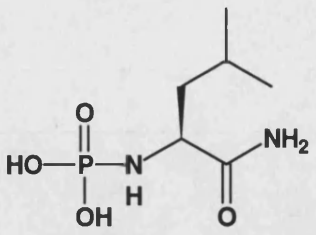
## 3.4 Generation of a Model for Ligand binding with Collagenase

Collagenase is a zinc-dependent endoproteinase and is a member of the matrix metalloproteinase (MMP)[282,283,284] family of enzymes. The MMPs are responsible for cleaving extracellular matrix components during normal connective tissue remodelling and are regulated by a wide range of factors, such as growth factors, hormones and the tissue inhibitor of the metalloproteinase (TIMP) family of proteins.[282,283]

The MMPs contain three regions: **i.** the $NH_2$ terminal propeptide (~80 residues) that contains a free cysteine thought to maintain enzyme latency by ligating to the catalytic zinc in the active site; **ii.** the catalytic domain which contains a zinc- and calcium-binding catalytic domain (~180 residues); and **iii.** a $CO_2H$ terminal domain (~200 residues) that may be involved in matrix binding[282] and alignment of the catalytic domain at the site of enzymatic cleavage.[283] Catalytic domains of the MMPs share a high degree of sequence homology. Early models of MMP's catalytic domains were based on the bacterial endoproteinase, thermolysin.[285]

Lovejoy *et al*[286,287] have discussed in detail the sequence homology of the active sites of collagenase and thermolysin. Both share three ligating ligands and a catalytic Glu. For collagenase there are three zinc-ligating residues (His 218, His 222 and His 228) and a catalytic zinc which share distinct homology with thermolysin (His 142, His 146 and Glu 166). Lovejoy *et al*[286,287] concluded that the Glu 143 (thermolysin) and Glu 219 (collagenase) act as a general base that together with the catalytic zinc, co-ordinates water molecules near the carbonyl of the scissile bond. Also, since collagenase is ligated by three neutral histidines rather than two His and a negatively charged Glu (as in thermolysin), collagenase catalytic zinc is predicted to have a higher net positive charge and a greater ability to stabilise the negative charge present in the transition-state than thermolysin in the catalytic zinc.

There are several crystal structures for thermolysin available on the Brookhaven Protein Database. These include bound phosporamidates, *e.g.* Leucinyl phosporamidate (**490**), which are believed to behave as transition-state analogues of the natural substrate, **Figure 98**.




**(490)**

**Figure 98**

Due to the high degree of homology between the two active sites, we were able to use the phosporamidate binding positions as a template for the binding position of the reduced peptide mimetic (**484**) to collagenase.

The co-ordinates for collagenase protein were supplied by Glaxo Group Research. We were given the data for a 10Å zone around the catalytic zinc.

### 3.4.1 Docking of the reduced peptide analogue (484) into Collagenase

The active sites of thermolysin and collagenase structures were superimposed using the metal centres, two histidines and the catalytic Glu. The natural peptide Ac-Ala-Leu-NHMe (**487**) was then manually docked into the active site and the conformation altered to fit that occupied by the phosporamidate in thermolysin. The natural peptide was minimised whilst inside the active site of collagenase which was kept fixed. The resultant natural peptide backbone was used for the conformation of the reduced peptide mimetic (**484**). The torsion angles for the minimised structure were $\phi_{ala}$ -161°, $\psi_{ala}$ -82°, $\phi_{leu}$ -39° and $\psi_{leu}$ -85°. This conformation lies inside a low energy region on both the Ala residue (**488**) and Leu residue (**489**) $\phi$, $\psi$ energy maps, which are 8 Kcal/mol above the global minimum for the charged species. However, for the uncharged species, the Ala residue lies in the second lowest energy region, and the Leu residue conformation lies at the energy minimum on the map.

Figure 99

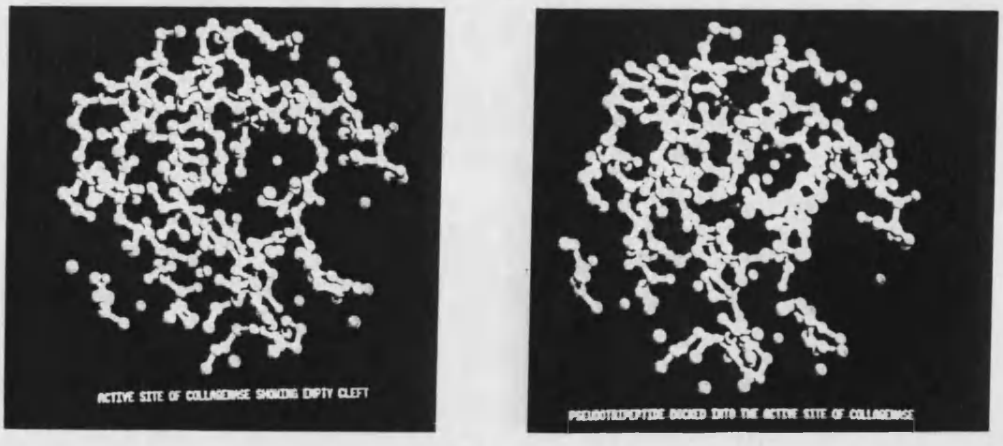



Figure 100

### 3.4.2 ΔG of Binding

The vibrational frequencies of a molecule are the dynamic properties. It is possible to calculate the harmonic vibrational frequencies of a molecule by considering infinitesimal displacements about a harmonic minimum. These may then be used to derive the vibrational free energy. It is possible to obtain information about the entropy and related thermodynamic properties of a conformation of a molecule from the vibrational frequencies. Using the vibrational and thermodynamic equations detailed by Dauber-Osguthorpe *et al*[288] the free energy, ΔG, of the isolated inhibitor and the bound, to the enzyme can be calculated. The reduced peptide mimetic (**484**) was minimised whilst in the active site. The active site was kept fixed, hence interactions between the mimetic (**484**) and the protein were included in the calculations, but the protein itself did not move.

The inhibitor was then removed and minimised again as the isolated molecule. The vibrational and thermodynamic data were calculated for the inhibitors both bound and isolated. From the resultant information, ΔS, ΔH, the ΔG for each was calculated and hence the ΔG of overall binding was evaluated.

Enthalpy,

$$H_{trans} = \frac{3}{2} RT$$

$$H_{rot} = \frac{3}{2} RT$$

Entropy,

$$S_{trans} = 2.303 \left( \frac{3}{2} \log_{10} M + \frac{5}{2} \log_{10} T - 0.5058 \right)$$

$$S_{rot} = 2.303 \left[ \frac{1}{2} \log_{10}(10^3 \gamma ABC) + \frac{3}{2} \log_{10} T - \log_{10} \sigma - 1.5072 \right]$$

where $\gamma = 47$ if the moment of inertia are evaluated in S.I. or M.K.S. units

ABC = the principle moments of inertia of non-linear molecules

$\sigma$ = symmetry value (in this case $\sigma = 1$ and therefore there is no contrabution)

Free Energy

$$\Delta G = \Delta H - T\Delta S$$

### 3.4.3 Results

When the charged inhibitor was used, the coulombic interactions were so great that the inhibitor was pushed out of the active site, resulting in the acetyl carbonyl binding to the catalytic zinc. When the uncharged inhibitor was used, this did not occur and the following results are shown in **Table 28.**

The energetics of binding are summarised in **Table 28.** A detailed summary of the procedure is given in the paper by Dauber-Osguthorpe *et al.*[281] The total intramolecular energy (valence and nonbond) of the pseudotripeptide is 8.5 kcal/mol, while the corresponding energy for the bound inhibitor is 31.6 kcal/mol. The total strain energy of binding is therefore 23.5 kcal/mol, reflecting the conformational constraints imposed by the enzyme on the inhibitor. However, this is more than compensated by the binding energy of -83.4 kcal/mol. A comparison of the energies and entropies are also shown in **Table 28.** The total change in free energy is ≈16kcal/mol which is due to the restriction of the inhibitor motion by the enzyme.

**Table 28.** Energy and entropy of pseudotripeptide **(485)**, isolated and bound in the active site of human Fibroblast collagenase collagenase. (Energies are in kcal/mol)

| Energy component | Isolated | Bound |
|---|---|---|
| Valence | | |
| Bond | 2.8 | 4.3 |
| Angle | 8.0 | 20.4 |
| Torsion | 0.8 | 4.0 |
| Out of plane | 0.0 | 0.9 |
| Cross terms | 1.1 | 1.3 |
| Total Valence | 12.7 | 30.9 |
| Intramolecular | | |
| VDW | 14.8 | 16.9 |
| Coulomb | -19.4 | -16.2 |
| Total nonbond | -4.6 | 0.7 |
| Total intramolecular | 8.1 | 31.6 |
| | | |
| Vibrational | | |
| Enthalpy | 261.7 | 263.2 |
| T x entropy | 26.4 | 20.3 |
| Free energy | 235.3 | 242.9 |
| | | |
| Rotational | | |
| Enthalpy | 0.9 | - |
| T x entropy | 3.9 | - |
| Free energy | -3.0 | - |
| | | |
| Translational | | |
| Enthalpy | 0.9 | - |
| T x entropy | 6.3 | - |
| Free energy | -5.4 | - |
| | | |
| Total | | |
| Enthalpy | 263.5 | 263.2 |
| T x entropy | 36.6 | 20.3 |
| Free energy | 226.9 | 242.9 |

# EXPERIMENTAL

## 4.1 Instrumentation and Experimental Techniques

### 4.1.1 Solvents and Reagents

All solvents were dried and distilled before use. Petrol refers to petroleum ether that boils in the range 60-80°C. Tetrahydrofuran and ether (refers to diethyl ether) were pre-dried over sodium wire and then refluxed over sodium benzophenone ketyl under a nitrogen atmosphere until anhydrous. These were redistilled immediately prior to use. Dichloromethane, dimethyl sulfoxide and triethylamine were distilled from calcium hydride. EtOAc was distilled from potassium carbonate. Methanol was distilled using the Lund and Bjerrum method.[289] All other solvents and reagents were purified using the procedures described in *Purification of Laboratory Chemicals.*[290]

### 4.1.2 Chromatography

Thin layer chromatography (t.l.c.) was used extensively as a qualitative guide during reactions and for assessing the purity of compounds. Whatman silica gel $F_{254}$ (0.25mm) sheets containing fluorescent indicator were used for this purpose. Components were visualised by illumination under sort wavelength (254nm) and ultraviolet light where possible. Plates were developed by treatment with 0.5% (w/v) aqueous solution of potassium permanganate, 7% (w/v) methanolic solution of phosphonmolybdic acid, 0.3% (w/v) ethanolic solution of ninhydrin, 4% (w/v) ethanolic solution of 2,4-dinitrophenylhydrazine and 3% (v/v) ethanolic solution of anisaldehyde, followed by warming of the t.l.c. plate.

Medium pressure flash chromatography was routinely employed using Amicon Matrex 60Å silica gel or alumina oxide, activated, neutral. Columns were packed as a slurry in the eluting solvent and the material to be chromatographed was

introduced directly as a solution in the eluting solvent or pre-absorbed onto the column support and then applied as a thin layer to the top of the column.

### 4.1.3 General

Glassware used for moisture sensitive reactions was heated in an oven at *ca.* 80°C and then allowed to cool in a desiccator over $CaCl_2$ and blue silca. Flasks and stirrer bars were additionally flame dried either under vacuum or a stream of dry nitrogen prior to use. Solvents were evaporated with a Büchi rotary evaporator using a water aspirator or vacuum pump as required, with the water bath temperature <35°C to avoid unnecessary heating.

### 4.1.4 Analysis and Spectroscopy

Melting points (m.p.) were determined using commercially available apparatus (Electrothermal Mk III, Gallenkamp and liquid thermalconducting melting point apparatus büchi 510) and were uncorrected. Elemental analysis was performed using a Carlo Erba 1106 Elemental analyser. Optical rotations were measured using a Perkin-Elmer 141 polarimeter with concentrations (*c*) expressed in $g/100cm^3$.

Infra-red spectra were recorded on a Perkin-Elmer 1600 series FT and 1310 infra-red spectrophotometer and peaks are reported ($\nu_{max}$) in wavenumbers $cm^{-1}$. Samples were prepared as liquid films, nujol mulls or $CHCl_3$ solutions.

Proton magnetic resonance spectra were recorded on a Joel JNM GX FT 270 (270MHz) spectrometer or on a Joel JNM GX FT 400 (400MHz) spectrometer where indicated . Carbon-13 magnetic resonance spectra were recorded on a Joel JNM GX FT 270 spectrometer operating at 67.8 MHz and using 90 and 135 DEPT pulse sequences to aid multiplicity determination. Chemical shifts (δ) are expressed in parts per million downfield of tetramethylsilane. The multiplicities of the resonances are denoted by s (singlet), d (doublet), t (triplet), q (quartet) and m

(multiplet) and the coupling constant values are expressed in Hz. The abbreviation br (broadened) is used to indicate significant broadening, whether due to rapid exchange or unresolved fine coupling. 2D homonuclear shift correlated spectroscopy (COSY) and nuclear Overhaüser effect spectroscopy (nOesy) were used to confirm proton assignments and conformational orientation when required.

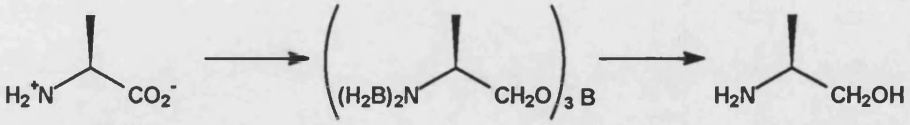
Mass spectra were recorded using a VG Analytical 7070E instrument with a VG 2000 data system. Electron (E.I.) spectra were produced using an ionising potential of 70 eV. Chemical ionisation (C.I.) was employed using isobutane as the reagent gas, although ammonia was also used where indicated. Accurate mass experiments were run on HP-engine/Autospec Q at Glaxo Group Research, Greenford.

## 4.2 Experimental Procedure

### 4.2.1 Phthaloyl protected compounds

**2(*S*)-Aminopropan-1-ol (344)**

To a stirred and cooled 0°C suspension of alanine (0.3g, 3.37mmol) in THF (5ml) under $N_2$, was added 1M $BH_3$-THF (10.1ml, 10.1mmol). The reaction mixture was allowed to attain room temperature and stirred for 6 hours. After cooling to 0°C, 3M NaOH (2.02ml, 6.06mmol) was added dropwise to control the effervescence. The suspension was allowed to warm to room temperature and stirred for a further 7 hours. The pH was then increased to 11 by addition of NaOH pellets and the solution was saturated with $K_2CO_3$ until two phases were observed. The aqueous phase was extracted with ether (2 x 10ml), the organics were combined, dried ($Na_2SO_4$) and then concentrated *in vacuo* to give a crude colourless oil. This was flash chromatographed on silica, eluting with petrol:EtOAc (25:75) to yield aminoalcohol **(344)** (0.16g, 63%) as a colourless oil, $R_f$ [petrol:EtOAc (25:75)] 0.62; b.p. 75°C (11 mm Hg); $\nu_{max}/cm^{-1}$ $3250_s$ (OH and NH), $1600_s$ (C-O), $1450_{\delta as}$ (Me), 1395 and 1170; $\delta_H$(270 MHz, $CDCl_3$) 1.24 (3H, d, *J* 6.6, Me), 2.80 (1H, br s, OH), 3.01 (1H, m, CH), 3.51 (1H, dd, *J* 11.4 and 7.2, $CH_2OH$), 3.89 (1H, dd, *J* 11.6 and 3.3, $CH_2OH$) and 4.38 (1H, br s, $NH_2$) and 4.55 (1H, br s, $NH_2$).




**(345)** **(344)**

**(2*S*)-Phthaloylaminopropan-1-ol (346)**

Phthalic anhydride (1.99g, 13.45mmol) was added to 2-aminopropan-1-ol **(344)** (1.01g, 13.45mmol) under $N_2$. The mixture was refluxed at 140-150°C for 2.5

hours. After cooling the reaction mixture was dissolved in DCM (50ml), washed with water (2 x 25ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to give a colourless crystalline solid (2.45g). This was flash chromatographed on silica, eluting with petrol:EtOAc (80:20), gradient up to petrol:EtOAc (50:50) to yield **(346)** (1.53g, 55%) as colourless crystals, $R_f$[petrol:EtOAc (50:50)] 0.29; m.p. 87° C (lit.,[145a] 86-87°C from acetone : hexane); $\nu_{max}/cm^{-1}$ $3485_s$ (OH) and $1687_s$ (C=O); $\delta_H$(270 MHz, $CDCl_3$) 1.41 (3H, d, *J* 7.2, Me), 2.80 (1H, br s, OH), 3.87 (1H, dd, *J* 7.9 and 3.9, C*H*$_2$OH), 4.03 (1H, dd, *J* 7.9 and 3.9, C*H*$_2$OH), 4.51 (1H, m, CH), 7.71 (2H, m, phthaloyl) and 7.82 (2H, m, phthaloyl); m/z (C.I.) 206 ($M^+$+1, 100%).


**(344)** **(346)**

**(2*S*)-Phthaloylaminopropanoic acid (348)**

**Method A**

Phthalic anhydride (32.91g, 220mmol) was added to alanine (20.1g, 226mmol). The mixture was heated to 120°C and stirred for 7 hours. After cooling the colourless solid was dissolved in EtOAc (500ml), washed with water (3 x 500ml) to remove any remaining alanine. The organics were dried ($MgSO_4$) and concentrated *in vacuo* to yield the protected amino acid **(348)** in quantitative yield as a colourless solid (46.38g), $R_f$ [DCM:MeOH (90:10)] 0.65; m.p. 148-150°C (lit.,[145a] 150-151° C from water); $[\alpha]_{589}^{20}$ -24.7 (*c* 1.25 in EtOH) [lit.,[145a] -25.12 (*c* 8.12 in EtOH)]; $\delta_H$(270 MHz, $CDCl_3$) 1.72 (3H, d, *J* 7.2, Me), 5.05 (1H, q, *J* 7.2, CH), 7.74 (2H, m, phthaloyl), 7.86 (2H, m, phthaloyl), 11.35 (1H, br s, $CO_2H$).


**(345)** **(348)**

The reaction was also carried out at 170°C for 2 hours and worked up as before to yield the protected amino acid in quantitative yield as a colourless solid, $R_f$ [DCM:MeOH (90:10)] 0.65; m.p. 147-149°C (lit.,[145a] 150-151°C from water); $[\alpha]_{589}^{20}$ -5.6 (*c* 0.753 in EtOH) [lit.,[145a] -25.12 (*c* 8.12 in EtOH)].

The reaction was also carried out at 140°C for 6 hours and worked up as before to yield the protected amino acid in quantitative yield as a colourless solid, $R_f$ [DCM:MeOH (90:10)] 0.65; m.p. 148-149°C (lit.,[145a] 150-151°C from water); $[\alpha]_{589}^{20}$ -9 (*c* 1.008 in EtOH) [lit.,[145a] -25.12 (*c* 8.12 in EtOH)].

The reaction was also carried out at 130°C for 5 hours and worked up as before to yield the protected amino acid in quantitative yield as a colourless solid, $R_f$ [DCM:MeOH (90:10)] 0.65; m.p. 148-149°C (lit.,[145a] 150-151°C from water); $[\alpha]_{589}^{20}$ -23.3 (*c* 1.218 in EtOH) [lit.,[145a] -25.12 (*c* 8.12 in EtOH)].

**Method B**

To a solution of alanine (30.55g, 343mmol) and phthalic anhydride (50.36g, 340mmol) in toluene was added triethylamine (4.8ml, 34mmol). The reaction mixture was refluxed for 3 hours removing the water with a Dean-Stark apparatus (*ca.* 6ml). Water (500ml) was poured into the reaction mixture and the product was allowed to crystallise. The crude product was filtered and dissolved in EtOAc (1000ml), washed with water (3 x 700ml), dried ($MgSO_4$) and concentrated *in vacuo* to yield the protected amino acid **(348)** (70.2g, 93%) as colourless crystals; $R_f$ [DCM:MeOH (90:10)] 0.65; m.p. 142-145°C (lit.,[152] 150-151°C from water); $[\alpha]_{589}^{20}$ -24.3 (*c* 2.63 in EtOH) [lit.,[152] -24.2 (*c* 2.6 in EtOH)].

**Preparation of (2*S*)-phthaloylaminopropan-1-ol (346) by reduction of (348)**

**Method A**

To a cooled (0°C) solution of the protected amino acid **(348)** (19.73g, 90mmol) in THF (200ml) was added 1M $BH_3$-THF (100ml, 100mmol). The reaction mixture was allowed to stir at room temperature for 2 days before quenching with water (300ml), extracted with EtOAc (2 x 300ml), the organics were washed with saturated $NaHCO_3$ (2 x 200ml), brine (200ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was flash chromatographed on silica, eluting with petrol:EtOAc (40:60) to yield the alcohol **(346)** (16.6g, 88%) as colourless crystals; $R_f$ [petrol:EtOAc (75:25)] 0.75; m.p. 87°C (EtOAc) (lit.,[145a] 86-87°C from acetone : hexane); $[\alpha]_{589}^{20}$ +12.5 (*c* 4.1 in $CHCl_3$) [lit.,[145a] +13.7 (*c* 4.2 in $CHCl_3$)].


**(348)** **(346)**

**Method B**

To the protected amino acid **(348)** (19.73g, 90mmol) in THF (200ml) was added 1M $BH_3$-THF (100ml, 100mmol). The reaction mixture was stirred at 40°C for 2 hours before quenching with water (300ml) and extracted with EtOAc (200ml). The organics were washed with saturated $NaHCO_3$ (2 x 200ml), brine (200ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 17.6g of the crude product. This was flash chromatographed on silica, eluting with petrol:EtOAc (75:25) to yield the alcohol **(346)** (14.4g, 78%) as colourless crystals; $R_f$ [petrol:EtOAc (50:50)] 0.29; m.p. 87°C ( from EtOAc) (lit.,[145a] 86-87°C from acetone : hexane).

**Method C**

To a cooled (0°C) solution of the protected amino acid **(348)** (23.9g, 110mmol) in THF (150ml) was added 2M BMS (60ml, 120mmol). The reaction mixture was allowed to stir at room temperature for 2 days before quenching with water (300ml) and extracting with EtOAc (200ml). The organics were washed with saturated $NaHCO_3$ (2 x 200ml), brine (200ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was flash chromatographed on silica, eluting with petrol:EtOAc (40:60) to yield the alcohol **(346)** (14.2g, 63%) as colourless crystals; $R_f$ [petrol:EtOAc (75:25)] 0.75; m.p. 87°C (EtOAc) (lit.,[145a] 86-87°C from acetone : hexane).

**(2*S*)-Phthaloylaminopropan-1-al (350)**

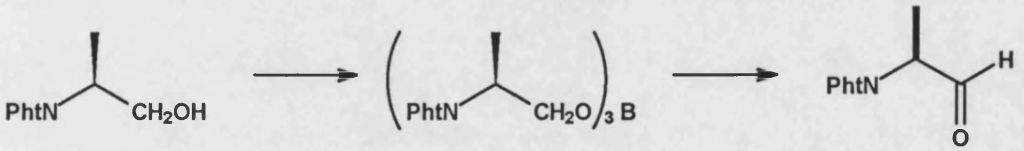
**Method A** :- General procedure

To a solution of alcohol **(346)** (1.5g, 7.3mmol) in DCM (80ml) under $N_2$ was added PCC (3.2g, 14.6mmol). The reaction mixture was refluxed for 3½ hours. After cooling, the reaction mixture was diluted with ether (55ml) and filtered through activated charcoal and celite. The filtrate was concentrated *in vacuo* and flash chromatographed through a short column eluting with EtOAc to afford aldehyde **(350)** (1.15g, 78%) as a colourless solid, m.p. 112°C (lit.,[145a] 112-113°C from acetone : hexane): $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25; $[\alpha]_{589}^{18}$ 0 (*c* 2.74 in $C_6H_6$) [lit.,[145a] -29.9 (*c* 2.16 in $C_6H_6$)]; (Found: C, 64.10; H, 4.15; N, 6.74, calc. for $C_{11}H_9NO_3$ : C, 65.02; H, 4.46; N, 6.89%); $\nu_{max}/cm^{-1}$ 1600, 1450, 1395, 1170; $\delta_H$(270 MHz, $CDCl_3$) 1.62 (3H, d, *J* 7.3, Me), 4.76 (1H, q, *J* 7.3, CH), 7.77 (2H, m, phthaloyl), 7.89 (2H, m, phthaloyl), 9.70 (1H, s, CHO); $\delta_C$ (67.8 MHz, $CDCl_3$) 12.90 (Me), 54.05 (*C*HMe), 123.61 (*m*-C), 131.83 (*C*C=O), 134.40 (*o*-C), 167.58 (N*C*=O) and 196.95 (*C*HO); m/z (70 eV) 174 (100%, M-CHO), (C.I) 204 ($M^+$+1, 100%).


**(346)** **(350)**

**Method B**

10-12M BMS (7.9ml, 79mmol) was added dropwise to a solution of the amino acid **(346)** (16.8g, 79mmol) in THF. After 4ml had been added the reaction mixture was heated to 40°C and the addition continued. The reaction was allowed to stir for 1 hour before cooling and concentrating *in vacuo* to give the crude trialkoxyboroxine **(355)**. This was dissolved in DCM (30ml), added to a vigorously stirred suspension of PCC (18.75g, 87mmol) in DCM (150ml) and refluxed for 4 hours. After cooling, the reaction mixture was diluted with ether (300ml) and filtered through activated charcoal and celite. The filtrate was concentrated *in vacuo* and flash chromatographed through a short column, eluting with EtOAc, afforded aldehyde **(350)** (7.0g, 48%) as a colourless solid, m.p. 112°C (lit.,[145a] 112-113°C from acetone : hexane): $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25.




**(346)** **(355)** **(350)**

**Method C**

To a solution of alcohol **(346)** (2.83g, 13.8mmol) in DCM (60ml) under $N_2$ was added celite (7.5g) and PCC (7.43g, 34.5mmol). After refluxing for 3 hours the reaction mixture was allowed to cooled before being diluted with ether (300ml). This was then filtered through Florisil (300g) and the residue washed with ether (4 x 200ml). The combined organics were concentrated *in vacuo* and recrystallised from EtOAc : hexane to afford aldehyde **(350)** (0.733g, 25%) as a colourless solid,

m.p. 110°C (lit.,[145a] 112-113°C acetone:hexane): $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25; $[\alpha]_{589}^{20}$ 0 (*c* 2.74 in $C_6H_6$) [lit.,[145a] -29.9 (*c* 2.16 in $C_6H_6$)].

**Method D**

To a solution of alcohol **(346)** (7.03g, 34.3mmol) in DCM (200ml) under $N_2$ was added alumina (45g) and PCC (21g, 97mmol). After stirring vigorously at 60°C for 30 minutes the reaction mixture was allowed to cool before being diluted with ether (300ml). This was then filtered through alumina (300g) and the residue was washed with ether (4 x 200ml), the combined organics were concentrated *in vacuo* and flash chromatographed, eluting with petrol:EtOAc (35:65) to afford aldehyde **(350)** (6.2g, 90%) as a colourless solid, m.p. 112°C (lit.,[145a] 112-113°C from acetone:hexane): $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25.

**Table 29**

| Alcohol (mmol) | support agent | equiv.s | crude yield (%) isolated % | time (hours) | temp. °C |
|---|---|---|---|---|---|
| 7 | none | 5 | 78 | 24 | 21 |
| 25 | none | 5 | 50 | 24 | 21 |
| 94 | none | 5 | 15 | 24 | 21 |
| 15 | none | 2 | (70) | 5 | 60 |
| 20 | none | 2 | 46 | 2 | 60 |
| 97 | alumina | 2.8 | 90 | 2½ | 60 |
| 29 | celite | 2 | (94) | 3 | 60 |
| 39 | celite | 2 | 75 | 3 | 60 |

**Method E**

A solution of oxalyl chloride (3.4ml, 37mmol) in DCM (75ml) was cooled to -78°C. To this was added DMSO (5.72ml, 74mmol) in DCM (20ml) at such a rate as to

maintain the temperature between -60 to -50°C. The reaction was then stirred for 2 minutes before the addition of the alcohol **(346)** (7.0g, 34mmol) in DCM (35ml). The reaction mixture was then stirred for 20 minutes at -78°C before the addition of triethylamine (23.8ml, 170mmol). After 5 minutes the reaction mixture was allowed to attain room temperature and then quenched with water (20ml), washed with 1% HCl (100ml), water (100ml), 5% $Na_2CO_3$ (100ml), water (100ml) and concentrated *in vacuo* to afford aldehyde **(350)** (6.2g, 90%) as a colourless solid, m.p. 111-112°C (lit.,[145a] 112-113°C from acetone:hexane); $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25.

**Method F**

To a solution of alcohol **(346)** (0.265g, 1.29mmol) in DCM (2.6ml) under $N_2$ was added NMO (0.227g, 1.94mmol), powdered 4Å molecular sieves and TPAP (25mg, 0.07mmol). After stirring at room temperature for 2 hours, starting material still remained and by-products were also beginning to form. The reaction mixture was flash chromatographed, eluting with petrol:ether (65:35) to afford the aldehyde **(350)** (0.93g, 35%) as a colourless solid, m.p. 112°C (lit.,[145a] 112-113°C from acetone:hexane): $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25. Alcohol **(346)** was also recovered (0.138g, 52%).

**Method G**

To a solution of alcohol **(346)** (1.311g, 6.4mmol) in DMSO (20ml) under $N_2$ was added pyridine-$SO_3$ (3.06g, 19.2mmol) in DMSO (20ml) and diisopropylethylamine (DIPEA) (7.25ml, 41.6mmol). After stirring at room temperature for 2 hours a negligible amount of the aldehyde was formed. The reaction mixture was diluted with ether (100ml) was with 1% HCl (80ml), water (100ml), the aqueous phase was back extracted with ether (150ml). The organics were combined, washed with sat. $NaHCO_3$ (80ml), water (2 x 80ml), brine (80ml), dried ($MgSO_4$) and concentrated

*in vacuo* to give alcohol **(346)** and aldehyde **(350)** (95:5) (1.22g, 93%) as a colourless solid.

**Method H**

To a cooled to -63°C solution of oxalyl chloride (1.43ml, 17.0mmol) in DCM (15ml) was added DMSO (1.76ml, 22.8mmol) in DCM (10ml) over a 15 minute period. To this was added the alcohol **(346)** (2.34g, 11.4mmol) in DCM (80ml) over 15 minutes. The reaction mixture was then stirred for 10 minutes at -63°C before the addition of DIPEA (7.94ml, 45.56mmol) over 15 minutes. The reaction mixture was allowed to stir for 15 minutes before being quenched with water (1.5ml/mmol). The reaction mixture was then added to pentane (150ml) and washed with 1% HCl (80ml), water (100ml) and the aqueous phase back extracted with ether (150ml). The organics were combined, washed with sat. $NaHCO_3$ (80ml), water (2 x 80ml), brine (80ml), dried ($MgSO_4$) and concentrated *in vacuo* to afford aldehyde **(350)** (1.66g, 72%) as a colourless solid, m.p. 111°C (lit.,[145a] 112-113°C from acetone:hexane); $R_f$ [petrol:EtOAc (70:30)] 0.39-0.25; $[\alpha]_{589}^{24}$ -38.6 (*c* 2.778 in $C_6H_6$) [lit.,[145a] -29.9 (*c* 2.16 in $C_6H_6$)].

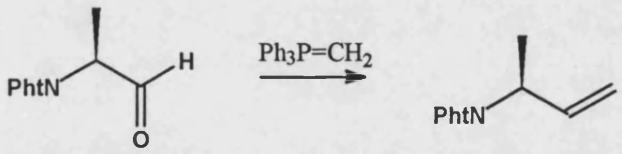

**(346)** **(350)**

***(3S)-Phthaloylaminobut-1-ene* (376)**

**Method A**

To a stirred suspension of methyltriphenylphosphonium bromide (0.854g, 2.39mmol) in THF (10ml) at -78°C, was added 1.6M *n*-BuLi (0.91ml, 2.28mmol) dropwise over the course of 5 minutes, upon which a bright yellow colour of the ylide was observed. The reaction mixture was then allowed to warm to room

temperature before stirring for 30 minutes. After this time the reaction mixture was cooled to -78°C before it was added to a cooled solution of the aldehyde **(350)** (0.442g, 2.17mmol) in THF (10ml) over the course of 30 minutes. A dark red-brown solution formed immediately on addition. The reaction mixture was allowed to warm to room temperature and stirred for 30 minutes. Over this period the dark colour slowly turned pale orange-yellow. Water (30ml) was added to the reaction, and the aqueous phase extracted with EtOAc (2 x 30ml). The organics were dried ($MgSO_4$) and concentrated *in vacuo* to give 0.44g of the crude product. This was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give the alkene **(376)** (0.17g, 38%); $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane); $[\alpha]_{589}^{22}$ +25.4 (*c* 3.2 in $CHCl_3$); (Found: C, 71.40; H, 5.77; N, 6.63, calc. for $C_{12}H_{11}NO_2$ : C, 71.63; H, 5.51; N, 6.96%); $\nu_{max}/cm^{-1}$ $1772_s$ (C=O), $1752_s$ (C=O), $1722_s$ (C=O), $1461_s$ (C=C) and 1377; $\delta_H$(270 MHz, $CDCl_3$) 1.58 (3H, d, *J* 7.1, Me), 4.94 (1H, qd, *J* 7.3 and 6.3, C*H*Me), 5.17 (1H, dt, *J* 10.3 and 1.4, C*H*H), 5.22 (1H, dt, *J* 17.2 and 1.4, CH*H*), 6.20 (1H, ddd, *J* 16.9, 10.3 and 6.6, C*H*=$CH_2$), 7.70 (2H, m, phthaloyl) and 7.82 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 18.16 (Me), 48.85 (*C*HMe), 116.31 (CH=*C*$H_2$), 123.06 (*m*-C), 131.95 (*C*C=O), 133.83 (*o*-C), 136.75 (*C*H=$CH_2$) and 167.9 (N*C*=O); m/z (70 eV) 201 ($M^+$, 60%), 186 (100, M-$CH_3$), low (eV) 201 ($M^+$, 100), 186 (50, M-$CH_3$).

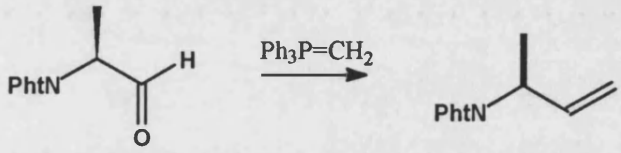



**(350)** **(376)**

**Method B**

To 60% NaH in mineral oil (1.084g, 21.7mmol) was added DMSO (5ml, 21.7mmol) the solution was heated to 75-80°C for 45 minutes. The resulting solution of methylsulfinyl carbanion was cooled to 0°C and methyltriphenylphosphonium bromide (10.77g, 29.6mmol) in DMSO (30ml) was

added. The resulting ylide was stirred for 10 minutes before being added to aldehyde **(350)** (4.0g, 19.7mmol) in THF (20ml). This was stirred at room temperature for 3 hours before quenching with water (200ml) and extracting with ether (5 x 100ml). The organics were combined and washed with brine (100ml), dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude product was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give alkene **(376)** (1.1g, 26%) as a colourless solid, $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane); $[\alpha]_{589}^{22}$ 0 (*c* 2.2 in $CHCl_3$).




**(350)** **(376)**

**Method C**

The alkene **(376)** was prepared in an analogous manner to method B using DMSO instead of THF. This gave alkene **(376)** (27%) as a colourless solid, $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane); $[\alpha]_{589}^{22}$ 0 (*c* 2.2 in $CHCl_3$).

**Method D**

To a stirred and cooled suspension (-20°C) of methyldiethylphosphonate (0.143ml, 0.98mmol) and 80% NaH (0.03g, 0.98mmol) in toluene (5ml) under $N_2$ was added a solution of the aldehyde **(350)** (0.2g, 0.98mmol) in toluene (5ml). The reaction was stirred for 30 minutes before being refluxed for 1 hour. After this time no desired product was observed by t.l.c. but many by-products were formed. The aldehyde **(350)** was not recovered and the reaction mixture not purified.

**Method E**

To a stirred and cooled (0°C) suspension of methyltriphenylphosphonium bromide (9.9g, 27.7mmol) in THF (80ml), was added 1.0M NaHMDS (27.5ml, 27.5mmol) dropwise over 5 minutes upon which a bright yellow colour of the ylide was observed. The reaction mixture was then allowed to warm to room temperature and stirred for 15 minutes. The ylide was added to a cooled solution (0°C) of the aldehyde **(350)** (1.126g, 5.54mmol) in THF (20ml) over 30 minutes. The reaction mixture was then allowed to warm to room temperature and stirred for 2 hours. Water (100ml) was added to the reaction, and the aqueous phase extracted with EtOAc (2 x 80ml). The organics were dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product which was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give the alkene **(376)** (0.061g, 5%); $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane).

**Method F**

To a stirred and cooled (-78°C) suspension of methyltriphenylphosphonium bromide (0.936g, 2.6mmol) in toluene (20ml), was added 1.6M *n*-BuLi (1.64ml, 2.6mmol) over 5 minutes upon which a bright yellow colour of the ylide was observed. The reaction mixture was then allowed to warm to room temperature before stirring for 15 minutes. After this time the reaction mixture was cooled to -78°C before it was added to a cooled solution of the aldehyde **(350)** (0.442g, 2.17mmol) in THF (10ml) over 30 minutes. A dark red-brown solution formed immediately on addition. The reaction mixture was allowed to warm to room temperature and stirred for 30 minutes. Over this period the dark colour slowly turned pale orange-yellow. Water (30ml) was added to the reaction, and the aqueous phase extracted with EtOAc (2 x 30ml). The organics were dried ($MgSO_4$) and concentrated *in vacuo* to give 0.751g of crude product which was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give the

alkene **(376)** (0.268g, 56%); $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane); $[\alpha]_{589}^{24}$ +25.4 (*c* 1.318 in $CHCl_3$).

**Method G**

To a stirred suspension of methyltriphenylphosphonium bromide (2.32g, 6.5mmol) in toluene (20ml) at room temperature, was added KO$^t$Bu (0.717g, 6.2mmol) and stirred for 15 minutes. After this time the reaction mixture was cooled (-20°C) before addition to a cooled (-20°C) solution of aldehyde **(350)** (1.2g, 5.9mmol) in toluene (20ml) over 30 minutes. An orange solution formed immediately on addition. The reaction mixture was allowed to warm to room temperature and stirred for 10 hours. Over this period the orange colour slowly turned to a pale yellow solution. Water (50ml) was added to the reaction, and the aqueous phase extracted with EtOAc (80ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 2.28g of crude product which was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give the alkene **(376)** (0.75g, 63%); $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane); $[\alpha]_{589}^{24}$ +12.0 (*c* 1.332 in $CHCl_3$).

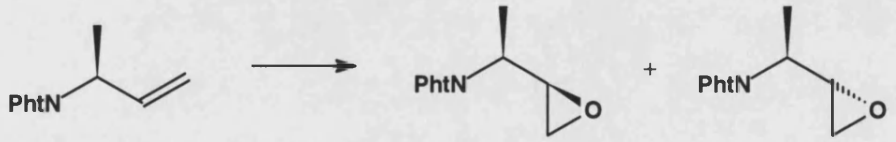
**Method H**

To a cooled (0°C) suspension of methyltriphenylphosphonium bromide (7.5g, 21mmol) in toluene (40ml), was added 1.0M NaHMDS (20ml, 20mmol) over 5 minutes upon which a bright yellow colour of the ylide was observed. The reaction mixture was warmed to room temperature, stirred for 20 minutes and then cooled to -78°C. This was added to a cooled solution (-78°C) of aldehyde **(350)** (4.35g, 20mmol) in toluene (40ml) over 30 minutes. A dark red-brown solution formed immediately on addition. The reaction mixture was stirred at -78°C for 10 minutes and then stirred for 4 hours at room temperature. Over this period the dark colour slowly turned pale orange-yellow. Water (100ml) was added to the reaction, and the aqueous phase extracted with ether (100ml), washed with brine, dried ($MgSO_4$)

and concentrated *in vacuo* to give 6.4g of crude product which was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give the alkene **(376)** (4.1g, 98%); $R_f$ [petrol:EtOAc (95:5)] 0.31; m.p. 84°C (recrystallised from cyclohexane); $[\alpha]_{589}^{21}$ +22.6 (*c* 1.386 in $CHCl_3$).

***(2R)* and *(2S)-[1(S)-Phthaloylaminoethyl] oxirane* (322) *and* (323).**

To a stirred solution of alkene **(376)** (0.428g, 21.3mmol) in DCM (200ml) was added *m*-CPBA (33.4g, 106.4mmol). The reaction mixture was left stirring for 2 days under $N_2$. The resulting mixture was then diluted with ether (300ml), washed with cooled (0°C) solution of 10% $Na_2SO_3$ (4 x 200ml), saturated $NaHCO_3$ (4 x 200ml) brine (200ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 5.32g of the crude epoxide. This was flash chromatographed on silica, eluting with petrol:EtOAc (70:30) to give **(322)** and **(323)** (63.5:36.5) (4.58g, 99%).




**(376)** **(322)** **(323)**

***(2R)-[1(S)-Phthaloylaminoethyl] oxirane* (322)**

Epoxide **(322)** (2.91g) was obtained as a colourless solid, $R_f$[petrol:EtOAc(70:30)] 0.45; m.p. 96°C (from EtOAc); $[\alpha]_{589}^{20}$ +24.5 (*c* 1.108 in $CHCl_3$); (Found C, 66.37; H, 5.1; N, 6.45. calc. for $C_{12}H_{11}NO_3$ : C, 66.35; H, 5.1; N, 6.45%); $\nu_{max}/cm^{-1}$ $1768_s$ (C=O), $1715_s$ (C=O), 1606 and $1254_s$ (C-O epoxide); $\delta_H$(270 MHz, $CDCl_3$) 1.64 (3H, d, *J* 7.0, Me), 2.61 (1H, dd, *J* 2.6 and 4.8, C*H*HO), 2.76 (1H, dd, *J* 3.9 and 1.1, CH*H*O), 3.54 (1H, ddd, *J* 7.3, 3.9 and 2.6, CHO), 3.97 (1H, dq, *J* 7.2 and 7.0, C*H*Me), 7.74 (2H, m, phthaloyl) and 7.85 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.63 (Me), 46.58 ($CH_2O$), 49.63 (*C*HMe), 51.93 (CHO), 123.03 (*m*-C),

$CDCl_3$) 14.63 (Me), 46.58 ($CH_2O$), 49.63 (*C*HMe), 51.93 (CHO), 123.03 (*m*-C), 131.69 (*C*C=O), 133.83 (*o*-C) and 167.85 (C=O); m/z (70eV) 217 ($M^+$, 0.5%), 174 (100, M-$CHCH_2O$), (C.I.) 218 ($M^+$+1, 100).

***(2S)-[1(S)-Phthaloylaminoethyl] oxirane* (323)**

Epoxide **(323)** (1.67g) was obtained as a colourless solid, $R_f$[petrol:EtOAc(70:30)] 0.55; m.p. 127-128°C (from EtOAc); $[\alpha]_{589}^{20}$ +10.7 (*c* 0.814 in $CHCl_3$) ; (Found C, 66.37; H, 5.1; N, 6.45. calc. for $C_{12}H_{11}NO_3$ : C, 66.35; H, 5.1; N, 6.45%); $\nu_{max}$/$cm^{-1}$ $1771_s$ (C=O), $1715_s$ (C=O), 1606 and $1254_s$ (C-O epoxide); $\delta_H$(270 MHz, $CDCl_3$) 1.52 (3H, d, *J* 7.2, Me), 2.75 (1H, dd, *J* 2.6 and 4.8, C*H*HO), 2.92 (1H, dd, *J* 3.8 and 0.9, CH*H*O), 3.62 (1H, ddd, *J* 7.3, 3.8 and 2.6, CHO), 4.07 (1H, dq, *J* 7.3 and 7.2, C*H*Me), 7.73 (2H, m, phthaloyl) and 7.84 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.86 (Me), 46.58 ($CH_2O$), 49.69 (*C*HMe), 52.58 (CHO), 123.29 (*m*-C), 131.82 (*C*C=O), 134.09 (*o*-C) and 167.98 (C=O); m/z (70eV) 217 ($M^+$, 0.5%), 174 (100, M-$CHCH_2O$), (C.I.) 218 ($M^+$+1, 100).

***General preparation of (2R)- and (2S)-[1(S)-phthaloylaminoethyl]oxirane* (322) *and* (323) *from aldehyde (350) using trimethylsulfur ylides (386, 387).***

**Method A**

To a stirred and cooled (0°C) suspension of trimethylsulfonium iodide (0.315g, 1.54mmol) in THF (5ml) was added 1.6M *n*-BuLi (0.88ml, 1.41mmol) dropwise. After addition the reaction mixture was stirred for 5 minutes at 0°C before aldehyde (350) (0.26g, 1.28mmol) in THF (5ml) was added dropwise. The colourless solution turned orange upon addition and was left to stir for 30 minutes before being allowed to attain room temperature (orange colour gradually faded). The reaction mixture was quenched with water (20ml) and concentrated *in vacuo*. The resulting solution was extracted with EtOAc (2 x 20ml), dried ($MgSO_4$) and

concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (30:70) to give **(322)** and **(323)** (68:32) (5.4mg, 2%).

**Method B**

Trimethylsulfoxonium chloride (3.7g, 28.77mmol) in THF (50ml) was added to 60% NaH (1.107g, 27.67mmol). The reaction mixture was heated at 65°C for 1¾ hours and then cooled to 55°C. Aldehyde **(350)** (4.5g, 22.1mmol) in THF (50ml) was added dropwise to this solution over an hour. The reaction mixture was stirred for a further hour at 55°C. After cooling, the reaction mixture was quenched with water (50ml) and extracted with EtOAc (5 x 100ml). The organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 3.5g of crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to yield **(322)** and **(323)** (55.4:44.6) (1.29g, 27%).

**Method C**

Trimethylsulfoxonium chloride (2.47g, 18.8mmol) in DMSO (10ml) was added to 60% NaH (0.752g, 18.8mmol) under $N_2$. This was stirred for 15 minutes at room temperature before the addition of the aldehyde **(350)** (3.2g, 15.7mmol) in DMSO (10ml). The reaction mixture was stirred for 30 minutes at room temperature before heating to 55°C for 5 hours. After cooling, the reaction mixture was quenched with water (50ml) and extracted with EtOAc (5 x 100ml). The organics were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to yield **(322)** and **(323)** (55.4:44.6) (0.92g, 27%).

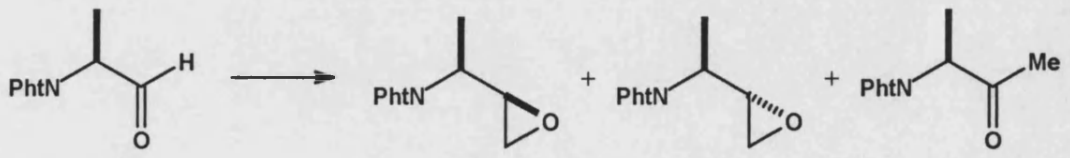
**Method D**

**Preparation of diazomethane**

Using the Aldrich diazald kit an ethereal diazomethane solution was prepared as follows:

Into a 250ml round bottom flask, fitted with a distillation head, water condensor, receiver adapter and collecting round bottom flask, was placed potassium hydroxide (6.2g, 0.11mol), water (10.5ml), ether (10.5ml) and ethanol (37ml). The distilling flask was heated in a water bath at 70-75°C with stirring ("Teflon" coated stirrer bar) and a solution of *N*-nitroso-*N*'-methyl-*p*-methyltoluene sulfonamide in ether (200ml) added at a rate approximately equivalent to the rate of the condensing distillate. Once all the nitrosamide solution had been added, additional ether was placed in the dropping funnel and added at the same rate until the distillate was colourless. The distillate contained 2.8-3.0g (64-69%) of diazomethane. For safety details see De Boer and Backer.[291]

Freshly distilled diazomethane (70mmol) was added to the aldehyde **(350)** (7g, 35mmol) in EtOAc (100ml). The reaction mixture was stirred at room temperature for 2 days before quenching with excess methanol (10ml). The reaction mixture was concentrated *in vacuo* and flash chromatographed on silica, eluting with petrol:EtOAc (70:30) to yield **(322)** and **(323)** (62:38) (0.582g, 8%). Also isolated was the corresponding methyl ketone **(393)** (3g, 40%):




**(350)** **(322)** **(323)** **(393)**

*(3S*)-Phthaloylaminobut-2-one* **(393)**

(3*S**)-Phthaloylaminobut-2-one **(393)** was isolated as a colourless solid, $R_f$[petrol:EtOAc (60:40)] 0.61; m.p. 78°C; (Found C, 66.02; H, 5.13; N, 6.49. calc. for $C_{12}H_{11}NO_3$ : C, 66.35; H, 5.10; N, 6.45%); $\nu_{max}/cm^{-1}$ $1775_s$ (C=O), $1697_s$ (C=O), $1465_{\delta\ as}$ (Me), 1378 and 714; $\delta_H$(270 MHz, $CDCl_3$) 1.67 (3H, d, *J* 7.3, Me), 2.22 (3H, s, COMe), 4.82 (1H, q, *J* 7.3, CH), 7.74-7.78 (2H, m, phthaloyl), 7.86-7.9 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 13.50 (Me), 26.40 ($CH_3O$), 54.85 (*C*HMe), 123.60 (*m*-C), 131.90 (*C*-CO), 134.30 (*o*-C), 168.00 (N*C*=O) and 203.00 (Me*C*=O); m/z (70 eV) 217 ($M^+$, 3%), 174 (100, M-$COCH_3$), (C.I) 218 ($M^+$+1, 100).

**Method E**

To a solution of aldehyde **(350)** (3.0g, 14.8mmol) and TBAI (0.055g, 0.15mmol) in DCM (30ml) was added 50% aqueous NaOH and trimethylsulfonium iodide (3.02g, 14.8mmol). The reaction mixture was stirred at 50°C for several hours. T.l.c. showed many spots and the reaction was abandoned.

***Attempted preparation of (2R*,3S*,2'S) and (2S*,3S*,2'S)-1-hydroxy-2-(O-methyl leucinyl)-3-phthaloylaminobutane* (324) *and* (325)**

**Method A**

To a solution of the epoxides **(322)** and **(323)** (0.122g, 0.56mmol) in anhydrous DCM (5ml) was added Leu-OMe.HCl (0.112g, 0.62mmol). The reaction mixture was stirred at room temperature overnight, after which time no reaction had occurred. Triethylamine (0.086ml, 0.62mmol) was added to the reaction mixture and after stirring for 5 hours, no addition to the epoxide was observed. $BF_3.OEt_2$ (0.069ml, 0.56mmol) was also added and after 1 hour no change was observed, with only starting materials being visible by t.l.c.

**Method B**

To a solution of Leu-OMe (0.097g, 0.672mmol) in anhydrous DCM (10ml) was added $BF_3.OEt_2$ (0.083ml, 0.67mmol) followed by epoxide **(322)** (0.1g, 0.46mmol) after 20 seconds. The reaction mixture was stirred at room temperature for 5 hours, after which time two new compounds were seen by t.l.c. The reaction was diluted with EtOAc (15ml), washed with water (10ml), back extracted with EtOAc (4 x 15ml), dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (50:50) with gradient to EtOAc, to give the epoxide **(322)** (0.012g, 12%), diols **(338:337**, 1:3) (0.04g, 37%) and a uv and ninhydrin visible compound which decomposed before characterisation.

**Method C**

To a solution of Leu-OMe (0.131g, 1.4mmol) in anhydrous DCM (5ml) was added $BF_3.OEt_2$ (0.109ml, 1.38mmol) followed by epoxides **(322)** and **(323)** (0.193g, 0.89mmol) after 20 seconds. The reaction mixture was stirred at room temperature for ½ hour, after which time a new compound was observed by t.l.c. The reaction was concentrated *in vacuo* to give 0.433g of crude material which was flash chromatographed on silica, eluting with petrol:EtOAc (50:50) with gradient to EtOAc, to give epoxides **(337)** and **(338)** (0.16g, 83%) and $BF_3$-Leu-OMe **(397)** (0.092g, 31%).

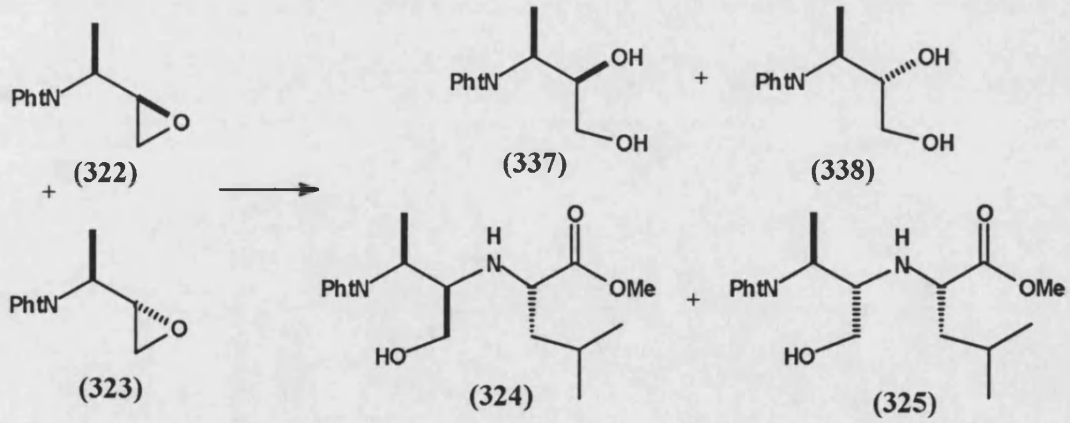
***$BF_3$-Leu-OMe* (397)**

$BF_3$-Leu-OMe **(397)** was isolated as a colourless oil; $\delta_H$(270 MHz, $CDCl_3$) 0.96 (6H, br s, $CHMe_2$), 1.70-1.80 (3H, m, $CH_2CH$), 3.80 (3H, s, OMe), 4.10 (1H, m, $CHCO_2Me$) and 6.45 (br s, $NH_2$); m/z (C.I.) 214 ($M^+$+1, 4%).


**(397)**

**Method D**

To a solution of epoxides **(322)** and **(323)** (0.16g, 0.74mmol) in anhydrous DCM (5ml) at room temperature was added $BF_3.OEt_2$ (0.092ml, 0.75mmol). A creamy white precipitate formed immediately, but disappeared on the addition of Leu-OMe (0.215g, 1.48mmol). The reaction mixture was stirred at room temperature for ½ hour, after which time new compounds were seen by t.l.c. The reaction was washed with 0.1M HCl (7.4ml), back extracted with DCM (5ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:acetone (30:70) to give a mixture of diols **(337)** and **(338)** and hydroxyl dipeptides **(324a)** and **(324b)**. These were recombined and flash chromatographed, eluting with petrol:EtOAc (80:20) to give the diols **(338:337**, 56:44) (0.004g, 2%), a mix of the diols **(338:337**, 20:80) and the hydroxymethyl dipeptide **(324b)** (0.04g) and the hydroxymethyl dipeptide **(324a)** (0.03g, 12%).



***(2R,3S,2'S)-2-Hydroxy-1-(O-methyl leucinyl)-3-phthaloylaminobutane* (324a)**

(2*R*,3*S*,2'*S*)-2-Hydroxy-1-(*O*-methyl leucinyl)-3-phthaloylaminobutane **(324a)** was isolated as a colourless gum; $R_f$ [petrol:EtOAc (25:75)]; 0.41; $\nu_{max}/cm^{-1}$ $3400_s$ (OH and NH), $1750_s$ (C=O), $1670_s$ (C=O), 1480 and 1390; $\delta_H$(400 MHz, $CDCl_3$) 0.91 (3H, d, *J* 6.4, $CH_2$*Me*), 1.03 (3H, d, *J* 6.4, $CH_2$*Me*), 1.52 (3H, d, *J* 7.1, CH*Me*), 1.70-1.87 (1H, m, $CH_2CHMe_2$), 1.90-1.99 (2H, m, $CH_2CHMe_2$), 2.60 (1H, brs, OH), 3.49 (1H, d, *J* 6.4, C*H*HOH), 3.52 (1H, d, *J* 5.2, CH*H*OH), 3.74 (3H, s, OMe), 3.89-3.95 (1H, m, CHN), 4.87 (1H, qd, *J* 7.1 and 4.4, C*H*Me), 5.08 (1H, dd, *J* 7.9 and 6.0, $CHCO_2Me$), 7.67-7.72 (2H, m, phthaloyl), 7.87-7.90 (1H, m, phthaloyl) and 7.90-7.94 (1H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.47 (Me), 21.70 and 22.93 ($CHMe_2$), 25.10 ($CHMe_2$), 43.72 ($CH_2CHMe_2$), 49.24 (NCHMe), 52.51 (OMe), 59.97 ($NCHCO_2$), 64.93 ($CH_2OH$), 73.78 (CHN), 124.23 (*m*-C), 125.23 (*m*-C), 129.16 (*C*C=O), 132.63 (*o*-C), 133.57 (*o*-C), 167.66 (C=O) and 172.56 ($CO_2Me$); m/z (70eV) 362 ($M^+$, 5%), 347 (5, M-$CH_3$), 331 (5, M-$CH_2OH$); (C.I.) 363 ($M^+$+1, 100).

***(2S,3R,2'S)-1-Hydroxy-2-(O-methyl leucinyl)-3-phthaloylaminobutane* (324b)**

(2*S*,3*R*,2'*S*)-1-Hydroxy-2-(*O*-methyl leucinyl)-3-phthaloylaminobutane **(324b)** was isolated as a colourless gum; $R_f$ [petrol:EtOAc (25:75)]; 0.51; $\nu_{max}/cm^{-1}$ $3400_s$ (OH and NH), $1730_s$ (C=O), $1650_s$ (C=O), $1460_{\delta\ as}$ (Me) and 1380; $\delta_H$(270 MHz, $CDCl_3$) 0.91 (3H, d, *J* 6.6, $CH_2$*Me*), 1.03 (3H, d, *J* 6.6, $CH_2$*Me*), 1.54 (3H, d, *J* 7.1, CH*Me*), 1.66-1.80 (1H, m, $CH_2CHMe_2$), 1.90-1.96 (2H, m, $CH_2CHMe_2$), 3.40-3.53 (2H, m, $CH_2OH$), 3.77 (3H, s, OMe), 3.85-3.96 (1H, m, CHN), 4.53 (1H, brs, OH), 4.97 (1H, qd, *J* 7.1 and 3.9, C*H*Me), 4.81 (1H, d, *J* 6.41, NH), 5.09 (1H, dd, *J* 7.3 and 6.4, $CHCO_2Me$), 7.68-7.74 (2H, m, phthaloyl), 7.82-7.85 (1H, m, phthaloyl) and 7.90-7.95 (1H, m, phthaloyl); m/z (70eV) 362 ($M^+$, 5%), 347 (5, M-$CH_3$), 331 (M-$CH_2OH$); (C.I.) 363 ($M^+$+1, 100).

**Method E**

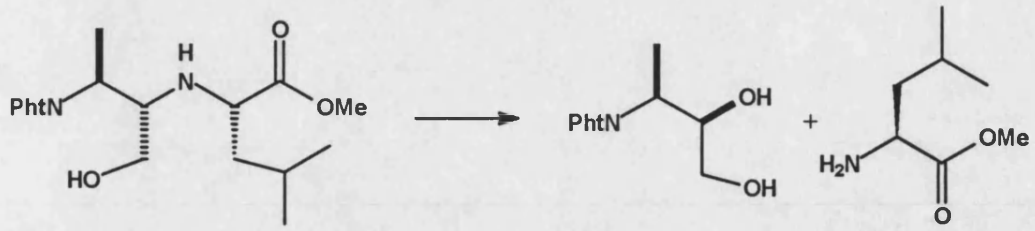
This reaction was performed using the same procedure as method D, but at 0°C. From the epoxides **(322)** and **(323)** (0.094g, 0.43mmol) flash chromatography of the crude material, eluting with petrol:EtOAc (65:35) gave the diols **(337)** and **(338)** (0.003g, %), a mixture of diols **(337)** and **(338)** and hydroxymethyldipeptide **(324b)** (0.081g) and pure hydroxymethyldipeptide **(324a)** (0.023g, 15%).

**Method F**

This reaction was performed using the same procedure as method D, but at -20°C. From epoxide **(322)** (0.215g, 0.99mmol) flash chromatography of the crude material, eluting with petrol:EtOAc (60:40) gave recovered epoxide **(322)** (0.074g, 21%), a mix of diols **(338:337**, 20:80) and hydroxymethyldipeptide **(325)** (0.07g) and pure hydroxymethyl dipeptide **(324)** (0.1g, 27%).

**Method G**

This reaction was performed using the same procedure as method F. From epoxide **(323)** (0.74g, 3.41mmol) flash chromatography of the crude material (1.1g) on neutral, activated, aluminium oxide, eluting with petrol:EtOAc (60:40) gave the reaction mixture unseparated. Flash chromatography of this material (0.8g) on silica, eluting with petrol:EtOAc (60:40) gave a mix of diols **(338:337**, 19:81) and hydroxymethyldipeptide **(325)** (0.27g) and pure hydroxymethyldipeptide **(324)** (0.04g, 3%). HPLC of the mixture of the diols **(337)** and **(338)** and hydroxymethyldipeptide **(325)** (0.05g), eluting with acetonitrile:water:TFA (20:80:1) gradient to 50:50:1 and back to 20:80:1 gave only the diols **(337)** and **(338)** and Leu-OMe. When the hydroxymethyldipeptide **(325)** was passed down the HPLC column only Leu-OMe and diol **(337)** were isolated.




**(325)** **(337)**

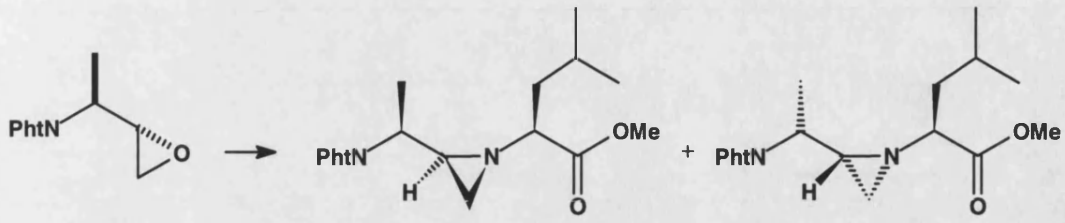
**Method H**

To a solution of the epoxides (**323:322**, 43:57) (0.278g, 1.28mmol) in anhydrous DCM (5ml) at -78°C was added $BF_3.OEt_2$ (0.173ml, 1.408mmol). A creamy white precipitate formed immediately, but this disappeared on the addition of Boc-Leu-OMe (0.345g, 1.408mmol). The reaction mixture was stirred at -78°C for ½ hour, after which time the reaction was allowed to attain room temperature and stirred for a further 20 minutes. After this time all the epoxides **(322)** and **(323)** had been consumed, as observed by t.l.c. The reaction was quenched with sat. ammonium chloride (1ml), washed with water (10ml), back extracted with EtOAc (10ml) and the combined organics dried ($MgSO_4$) and concentrated *in vacuo* to give 0.316g of crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (20:80) gradient to EtOAc to give Boc-Leu-OMe and a mixture of the diols (**338:337**, 9:91) (0.208g, 69%).

**Method I**

To a solution of epoxide **(323)** (0.095g, 0.44mmol) in anhydrous DCM (10ml) at -78°C was added $BF_3.OEt_2$ (0.057ml, 0.46mmol). A creamy white precipitate formed immediately and the reaction mixture was stirred at -78°C for 30 minutes before the addition of Leu-OMe (0.076g, 0.53mmol). The reaction mixture was stirred at -78°C for 5 hours, after which time another equivalent of Leu-OMe (0.076g, 0.53mmol) was added. The reaction was concentrated *in vacuo* and flash chromatographed on silica, eluting with petrol:EtOAc (20:80) to give recovered

epoxide **(323)** (0.033g, 35%) and a new compound believed to be the aziridine (**328a:328b**, ~1:1) (0.019g, 13%):




**(323)** **(328a)** **(328b)**

***(1R,2S,2'S)- and (1S,2R,2'S)-1-(Methyl-4'-methylpentanoate)-2-(1-phthaloyl-aminoethyl)aziridine* (328)**

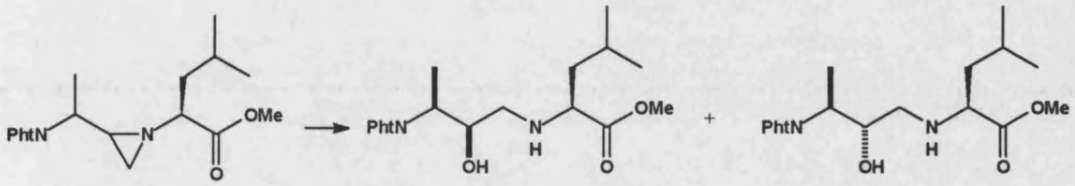
(1*R*,2*S*,2'*S*)- and (1*S*,2*R*,2'*S*)-1-(Methyl-4'-methylpentanoate)-2-(1-phthaloylamino-ethyl) aziridines **(328)** were isolated as a colourless gum; $R_f$ [petrol:EtOAc (70:30)]; 0.12; $\nu_{max}/cm^{-1}$ $1763_s$ (C=O), $1734_s$ (C=O), $1706_s$ (C=O), 1646, $1465_{\delta\ as}$ (Me), $1387_s$ (C-N), 1200 and 1172; $\delta_H$(270 MHz, $CDCl_3$) 0.85-0.95 (6H, m, CH*Me*$_2$), 1.57 (3H, d, *J* 7.2, CH*Me*), 1.42-1.50 (1H, m, C*H*Me$_2$), 1.60-1.80 (2H, m, C*H*$_2$CHMe$_2$), 2.40 (1H, dd, *J* 16.7 and 7.6, C*H*HN), 2.77 (1H, dd, *J* 16.7 and 5, CH*H*N), 3.24 (1H, ddd, *J* 7.6, 5 and 5, CHN), 3.60 and 3.66 (3H, s, OMe), 4.00-4.12 (1H, m, C*H*CO$_2$Me), 4.31-4.35 (1H, qd, *J* 6.9 and 6, C*H*Me), 7.70-7.78 (2H, m, phthaloyl) and 7.80-7.88 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.35 and 14.39 (Me), 22.03, 22.08, 22.76 and 22.80 (CH*Me*$_2$), 24.86 (*C*HMe$_2$), 42.44 and 42.55 (*C*H$_2$CHMe$_2$), 49.87 and 50.09 (N*C*HMe), 50.24 and 50.70 (CH$_2$N), 51.68 and 51.80 (OMe), 59.86 and 60.07 (N*C*HCO$_2$), 70.29 and 70.74 (CHN), 123.35 (*m*-C), 131.87 (*C*C=O), 134.13 (*o*-C), 168.61 (C=O) and 175.91 (*C*O$_2$Me).

and

$\delta_H$(270 MHz, $CDCl_3$) 0.85-0.95 (6H, m, CH*Me*$_2$), 1.57 (3H, d, *J* 7.2, CH*Me*), 1.42-1.50 (1H, m, C*H*Me$_2$), 1.60-1.80 (2H, m, C*H*$_2$CHMe$_2$), 2.46 (1H, dd, *J* 14.1 and 5, CH$_2$N), 2.69 (1H, dd, *J* 13 and 6.9, CH$_2$N), 3.24 (1H, dd, *J* 7.6, and 5,

CHN), 3.60 and 3.66 (3H, s, OMe), 4.00-4.12 (1H, m, C*H*$CO_2$Me), 4.31-4.35 (1H, qd, *J* 6.9 and 6, C*H*Me), 7.70-7.78 (2H, m, phthaloyl) and 7.80-7.88 (2H, m, phthaloyl);

Purification of (**328a:328b**, ~1:1) by HPLC eluting with acetonitrile:water:TFA resulted in the isolation of a different compound believed to be the hydroxyethylamino dipeptides (**398:399**, ~1:1):




(328) (398) (399)

***(2R*,3S*,2'S) and (2S*,3S*,2'S)-2-Hydroxy-1-(O-methyl leucinyl)-3-phthaloyl aminobutane* (398) *and* (399)**

Were isolated as a colourless oil; $\nu_{max}/cm^{-1}$ $3468_s$ (OH), $1760_s$ (C=O), $1734_s$ (C=O), $1706_s$ (C=O), 1646, $1465_{\delta\ as}$ (Me), $1387_s$ (C-N), 1200, and 1172; $\delta_H$(270 MHz, $CDCl_3$) 0.85-1.10 (6H, m, CH*Me*$_2$), 1.53 (3H, d, *J* 7.0, CH*Me*), 1.60-1.95 (3H, m, C*H*$_2$C*H*Me$_2$), 2.85-3.40 (2H, m, $CH_2N$), 3.80 and 3.85 (3H, s, OMe), 3.85-4.05 (1H, m, C*H*$CO_2$Me), 4.25-4.45 (1H, m, C*H*Me), 4.50-4.70 (1H, m, C*H*OH), 5.30-6.40 (2H, br s, NH and OH), 7.70-7.78 (2H, m, phthaloyl) and 7.80-7.88 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.16 and 14.25 (CH*Me*), 21.27, 21.35, 22.70 and 22.75 (CH*Me*$_2$), 24.77 and 24.81 (*C*HMe$_2$), 37.93 and 38.96 (*C*H$_2$CHMe$_2$), 49.56 ($CH_2N$), 49.67 and 49.83 (*C*HMe), 50.38 ($CH_2N$), 53.31 and 53.39 (OMe), 59.00 and 59.14 (*C*H$CO_2$), 67.68 and 68.21 (CHOH), 123.53 (*m*-C), 131.58 (*C*C=O), 134.35 (*o*-C), 168.49 and 169.26 (*C*ON), 172.00 (*C*$O_2$Me); m/z (FAB+) 363 ($M^+$+1); [Found : ($M^+$+1) 363.1945. $C_{19}H_{26}N_2O_5$ requires 363.1920].

**Method J**

To a cooled solution (-20°C) of the epoxides **(322)** and **(323)** (0.203g, 0.935mmol) in DCM (5ml) was added $BF_3.OEt_2$ (0.115ml, 0.935mmol). A creamy white precipitate formed immediately and the reaction mixture was stirred at -20°C for 1 minute before the addition of Bn-Leu-OMe **(367)** (0.264g, 1.12mmol). The reaction mixture was stirred at -20°C for 1 hour, after which time the reaction was quenched with water (0.25ml). The reaction was concentrated *in vacuo* and flash chromatographed on silica, eluting with petrol:EtOAc (20:80) to give recovered epoxides **(322)** and **(323)** (0.06g, 31%), Bn-Leu-OMe **(367)** and diols **(338:337**, 17:83) (0.06g, 28%).

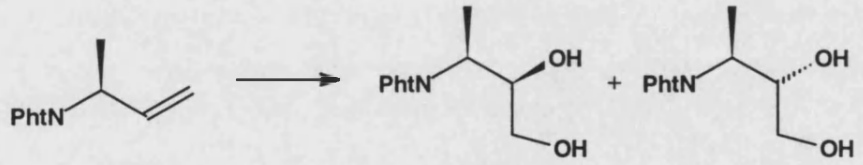
***(2S,3S,2'S)-1-Hydroxy-2-(O-methyl leucinyl)-3-phthaloylamino-butane*** **(325a)**

To a cooled solution (0°C) of the protected alcohol **(476)** (0.04g, 0.07mmol) in THF (1ml) was added TBAF (0.13ml, 0.13mmol). The reaction mixture was stirred for 2 hours, diluted with DCM (3 x 3ml), washed with brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude alcohol **(325a)**. These were flash chromatographed, eluting with petrol:EtOAc (40:60) to give the desired alcohol **(325a)** as a gum (0.015g, 67%); $R_f$ [$CHCl_3$:MeOH (90:10)] 0.13; $\nu_{max}/cm^{-1}$ $3400_s$ (OH and NH), $1750_s$ (C=O), $1670_s$ (C=O), 1480 and 1390; $\delta_H$(270 MHz, $CDCl_3$) 0.91 (3H, d, *J* 6.4, $CH_2$*Me*), 1.03 (3H, d, *J* 6.4, $CH_2$*Me*), 1.41 (3H, d, *J* 7.1, CH*Me*), 1.50-1.70 (1H, m, $CH_2CHMe_2$), 1.74-1.90 (2H, m, $CH_2CHMe_2$), 2.30-2.40 (1H, brs, OH), 3.60-3.70 (2H, m, $CH_2OH$), 3.78 (3H, s, OMe), 4.00-4.10 (1H, m, CHN), 4.67 (1H, qd, *J* 7.2 and 2.2, C*H*Me), 5.10 (1H, dd, *J* 7.5 and 6.4, $CHCO_2Me$), 7.67-7.72 (2H, m, phthaloyl), 7.87-7.90 (1H, m, phthaloyl) and 7.90-7.94 (1H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 13.03 (Me), 22.19 and 22.23 ($CHMe_2$), 24.92 ($CHMe_2$), 43.41 ($CH_2CHMe_2$), 49.17 (NCHMe), 52.28 (OMe), 60.03 ($NCHCO_2$), 63.89 ($CH_2OH$), 72.67 (CHN), 124.04 (*m*-C), 125.21 (*m*-C), 129.18 (*C*C=O), 132.49 and 132.71 (*o*-C), 133.42 and 134.26 (*o*-C), 167.66 and

168.82 (C=O) and 172.76 ($CO_2Me$); m/z (70eV) 362 ($M^+$, 5%), 347 (5, M-$CH_3$), 331 (5, M-$CH_2OH$); FAB + 363 ($M^+$+1, 60); FAB (-) 361 ($M^+$-1, 100).

***Preparation of the diols* (337) *and* (338) *using osmium tetraoxide***

To a cooled solution (0°C) of the alkene **(376)** (0.624g, 3.1mmol) in acetone:water:$^t$BuOH (6:5:1ml) was added NMO.$2H_2O$ (0.375g, 3.2mmol) and osmium tetraoxide (12.6mg, 0.03mmol). The reaction mixture was left to stir at room temperature for 16 hours. To this was added a slurry of sodium hydrosulfite (0.1g), magnesium silicate (1.2g) and water (8ml). This was filtered and the filtrate was neutralised with 1M $H_2SO_4$ and concentrated *in vacuo*. This was flash chromatographed on silica, eluting with petrol:EtOAc (20:80) to yield an inseparable mix of **(337)** and **(338)** (27:73) (0.324g, 44%):




**(376)** **(337)** **(338)**

***(2S,3S)-1,2-Dihydroxy-3-phthaloylaminobutane* (337)**

(2*S*,3*S*)-1,2-Dihydroxy-3-phthaloylaminobutane **(337)** was a colourless solid; $R_f$ [petrol:EtOAc (1:1)]; 0.26; (Found C, 61.14; H, 5.65; N, 5.87. calc. for $C_{12}H_{13}NO_4$ : C, 61.27; H, 5.57; N, 5.95%); $\nu_{max}/cm^{-1}$ $3439_s$ (OH), $1682_s$ (C=O) and $1062_s$ (C-O); $\delta_H$(270 MHz, $CDCl_3$) 1.47 (3H, d, *J* 7.1, CH*Me*), 2.40 (1H, br s, OH), 3.50 (1H, br s, OH), 3.56 (1H, m, C*H*HO), 3.68 (1H, m, CH*H*O), 4.10 (1H, m, CHO), 4.42 (1H, dq, *J* 7.1 and 4.8, C*H*Me), 7.75 (2H, m, phthaloyl) and 7.86 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 13.82 (Me), 48.78 (N*C*HMe), 63.86 ($CH_2OH$), 72.88 (CHOH), 123.45 (*m*-C), 131.72 (*C*C=O), 134.28 (*o*-C) and 168.83 (C=O);

m/z (70eV) 204 (28%, M-$CH_2OH$), 174 [100, M-$CH(OH)CH_2OH$]; (C.I.) 236 ($M^+$+1, 100).

*(2R,3S)-1,2-Dihydroxy-3-phthaloylaminobutane* (**338**)

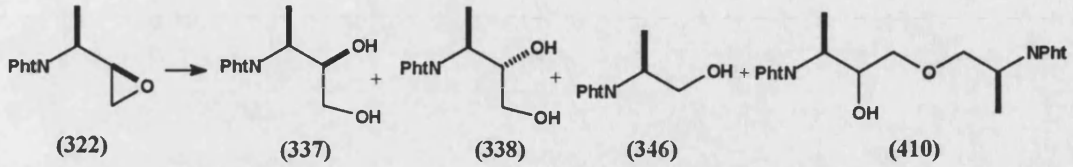
(2*R*,3*S*)-1,2-Dihydroxy-3-phthaloylaminobutane (**338**) was a colourless solid; $R_f$ [petrol:EtOAc (1:1)]; 0.26; (Found C, 61.14; H, 5.65; N, 5.87. calc. for $C_{12}H_{13}NO_4$ : C, 61.27; H, 5.57; N, 5.95%); $\nu_{max}/cm^{-1}$ $3447_s$ (OH), $1687_s$ (C=O) 1389 and $1064_s$ (C-O); $\delta_H$(270 MHz, $CDCl_3$) 1.48 (3H, d, *J* 7.1, CH*Me*), 2.40 (br s, OH), 3.50 (br s, OH), 3.56 (1H, dd, *J* 11.5 and 6.9, C*H*HO), 3.68 (1H, dd, *J* 12.5 and 4.0, CH*H*O), 4.12 (1H, ddd, *J* 7.1, 6.9 and 4.0, CHO), 4.56 (1H, dq, *J* 7.1 and 7.0, C*H*Me), 7.73 (2H, m, phthaloyl) and 7.83 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.37 (Me), 48.91 (N*C*HMe), 64.18 ($CH_2OH$), 72.75 (CHOH), 123.45 (*m*-C), 131.72 (*C*C=O), 134.12 (*o*-C) and 169.18 (C=O); m/z (70eV) 204 (28%, M-$CH_2OH$), 174 [100, M-$CH(OH)CH_2OH$]; (C.I.) 236 ($M^+$+1, 100).

***Ring opening of the oxiranes (322) and (323) to give the corresponding diols* (337) *and* (338)**

**Method A**

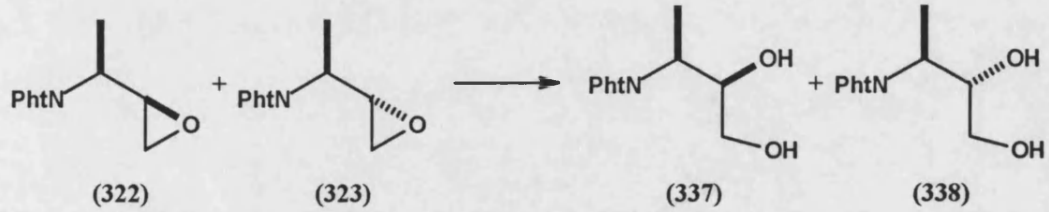
To a solution of the epoxide (**322**) (0.239g, 1.1mmol) in THF (1ml) and water (1ml) was added 1M $H_2SO_4$ (0.2ml). The reaction was allowed to stir at room temperature and monitored by t.l.c. After 3 hours the epoxide still remained and by-products began to form. After 5 hours the reaction was stopped as more by-products were observed. The reaction mixture was diluted with a sat. solution of $K_2CO_3$. The aqueous phase was extracted with EtOAc (3 x 15ml), the organics combined, washed with brine (30ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to give 0.19g of crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (50:50) to yield epoxides (**322**) and (**323**) (0.003g, 1%), alcohol

**(346)** (0.042g, 19%), and what appeared to be 2-phthaloylpropyloxy-(3-phthaloyl)-2-hydroxybutane **(410)** (0.023g, 5%) and diols [**(338:337)**, 7:93] (0.071g, 43%).




**Method B**

To a cooled solution (-20°C) of the epoxides (**323:322**, 43:57) (0.394g, 1.81mmol) in DCM (2ml) was added $BF_3.OEt_2$ (0.245ml, 2.0mmol). After 1 minute water (1ml) was added. The reaction was stirred at -20°C for 10 minutes before being warmed to room temperature and stirred for a further 2 hours. After this time no change occurred so the reaction was quenched with ammonium chloride (5ml) and extracted with $CHCl_3$ (3 x 10ml). The organics were dried ($Na_2SO_4$) and concentrated *in vacuo* to afford 0.395g crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (50:50) to give diols (**338:337**, 9:91) (0.136g, 35%) and recovered epoxides (0.104g, 26%).



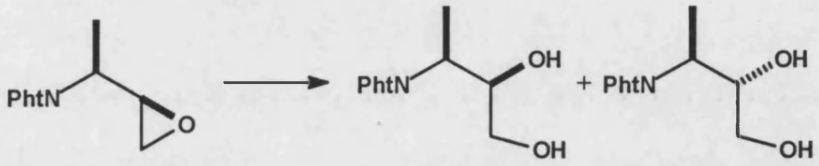

**Method C**

To a stirred solution of the epoxides **(322)** and **(323)** (0.506g, 2.33mmol) in THF (3ml) was added 0.1M $H_2SO_4$ (3ml). The reaction mixture was stirred at 50°C for 3 days, cooled, diluted with EtOAc (5ml), washed with sat. $NaHCO_3$, dried ($Na_2SO_4$) and concentrated *in vacuo* to afford 0.452g of crude material. This was

flash chromatographed on silica, eluting with petrol:EtOAc (40:60) to give diols (**338:337**, 9:91) (0.297g, 54%) and recovered epoxides (0.05g, 10%).

**Method D**

To a stirred solution of epoxide **(322)** (1.224g, 5.63mmol) in THF:water (10ml:10ml) was added Dowex-50X8-100 ion-exchange resin (strongly acidic). The reaction mixture was stirred at 50°C for 11 hours, cooled and filtered. The resin was washed with $CHCl_3$ (50ml), concentrated *in vacuo* and crystallised from hexane:EtOAc to afford diols **(337)** and **(338)** (0.73g, 56%).




**(322)** **(337)** **(338)**

**Method E**

To a solution of the epoxides (**323:322**, 35:65) (4.0g, 18.4mmol) in THF (100ml) was added 6% $HClO_4$ (100ml) and the reaction mixture stirred for 2 days. The reaction was diluted with DCM (200ml), washed with sat. $NaHCO_3$ until the pH was 8 and the aqueous phase back extracted with DCM (150ml). The organics were combined, dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (30:70) to give diols (**338:337**, 1:3) (2.88g, 66%).


**(322)** **(323)** **(337)** **(338)**

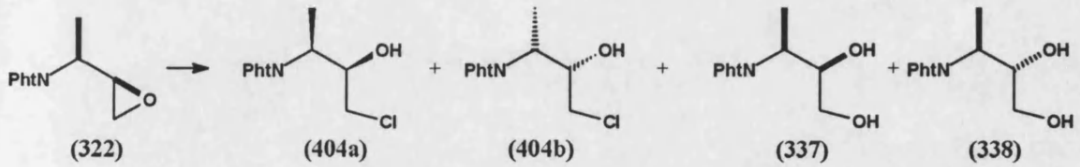
*Trifluoroacetyl-N-Leu-OMe* **(368)**

To a stirred suspension of Leu-OMe.HCl (0.58g, 3.2mmol) in THF (10ml) was added DIPEA (1.23ml, 7.04mmol) and TFAA (0.48ml, 3.36mmol). The reaction mixture was left to stir for 16 hours. The reaction mixture was filtered, diluted with EtOAc (20ml), washed with water (2 x 20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the protected product **(368)** (0.774g, 100%) as a yellow-orange oil; $R_f$ [petrol:EtOAc (80:20)] 0.2; (Found C, 44.27; H, 5.48; N, 5.67. calc. for $C_9H_{14}NF_3O_2$ : C, 44.28; H, 5.74; N, 5.66%); $\nu_{max}/cm^{-1}$ $3326_s$ (NH), $1751_s$ (C=O), $1717_s$ (C=O), $1564_s$ (NCO), 1440 and 1203; $\delta_H$(270 MHz, $CDCl_3$) 0.95 (6H, s, *J* 6.6, CH*Me*$_2$), 1.60-1.74 (2H, m, $CH_2$), 2.16 (2H, br s, C*H*Me$_2$), 3.79 (1H, s, OMe), 4.61-4.70 (1H, m, NCH) and 6.77 (1H, br s, NH); $\delta_C$ (67.8 MHz, $CDCl_3$) 21.95 (CH*Me*$_2$), 22.65 (CH*Me*$_2$), 24.86 (C*H*Me$_2$), 41.41 ($CH_2$), 51.23 (CHN), 52.83 (OMe), 113 and 118 ($CF_3$), 156 and 157 (CON) and 171.93 (*C*OMe); m/z (C.I.) 259 ($MNH_4^+$).

***(2S*,3S*) and (2R*,3S*)-1-Chloro-2-hydroxy-3-phthaloylaminobutane* (403) *and* (404)**

**Method A**

To a stirred solution of racemic epoxide **(322)** (0.219g, 1.01mmol) in 1,2-dimethoxyethane (4ml) and MeOH (1ml) was added tetraethylammonium chloride hydrate (0.9g, 5.1mmol). The mixture was refluxed for 3 days. After cooling the mixture was diluted with EtOAc (10ml), washed with 2M HCl (10ml), brine (10ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to afford 0.135g crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give chloroalcohol **(403)** (0.052g, 20%), chloroalcohol **(404)** (0.039g, 15%) and the racemic diols (**337:338**, 91:9) (0.05g, 16%);




(322) (404a) (404b) (337) (338)

***(2S*,3S*)-1-Chloro-2-hydroxy-3-phthaloylaminobutane* (403)**

(2*S**,3*S**)-1-Chloro-2-hydroxy-3-phthaloylaminobutane **(403)** was isolated as a colourless solid; $R_f$ [petrol:EtOAc(70:30)] 0.63; m.p. 87°C; (Found C, 56.66; H, 4.69; N, 5.40. calc. for $C_{12}H_{12}NO_3Cl$ : C, 56.82; H, 4.77; N, 5.52%); $\nu_{max}/cm^{-1}$ $3477_s$ (OH), $1688_s$ (C=O), $1376_{\delta\ s}$ (Me), 1133 and 1084; $\delta_H$(270 MHz, $CDCl_3$) 1.48 (3H, d, *J* 7.1, Me), 3.57 (1H, dd, *J* 11.4 and 5.0, C*H*HCl), 3.61 (1H, br s, OH), 3.68 (1H, dd, *J* 11.4 and 5.0, CH*H*Cl), 4.30-4.38 (1H, m, CHO), 4.68 (1H, dq, *J* 5.0 and 7.3, CHMe), 7.75-7.78 (2H, m, phthaloyl) and 7.87-7.90 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 13.52 (Me), 46.42 ($CH_2Cl$), 48.87 (*C*HMe), 72.68 (CHOH), 123.5 (*m*-C), 131.65 (*C*C=O), 134.33 (*o*-C) and 168.51 (C=O); m/z (70eV) 204 (7%,M-$CH_2Cl$), 174 (100, M-$HOCHCH_2Cl$); (C.I.) 254 ($M^+$+1, 100%).

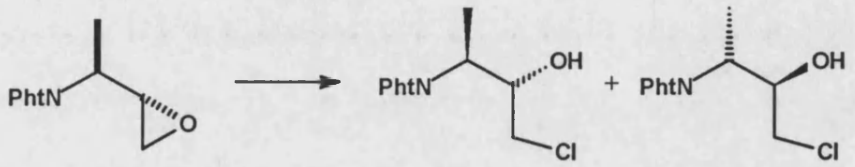
***(2R*,3S*)-1-Chloro-2-hydroxy-3-phthaloylaminobutane* (404)**

(2*R**,3*S**)-1-Chloro-2-hydroxy-3-phthaloylaminobutane **(404)** was isolated as a colourless solid; $R_f$[petrol:EtOAc (70:30)] 0.55; m.p. 119°C; (Found C, 56.50; H, 4.59; N, 5.32. calc. for $C_{12}H_{12}NO_3Cl$ : C, 56.82; H, 4.77; N, 5.52%); $\nu_{max}/cm^{-1}$ $3477_s$ (OH), $1688_s$ (C=O), $1462_{\delta\ as}$ (Me), $1376_{\delta\ s}$ (Me), 1133 and 1084; $\delta_H$(270 MHz, $CDCl_3$) 1.52 (3H, d, *J* 7.1, Me), 3.56 (1H, dd, *J* 11.5 and 5.0, C*H*HCl), 3.59 (1H, br s, OH), 3.66 (1H, dd, *J* 11.5 and 5.0, CH*H*Cl), 4.21-4.29 (1H, m, CHO), 4.69 (1H, dq, *J* 7.2 and 7.0, C*H*Me), 7.72-7.78 (2H, m, phthaloyl) and 7.82-7.88 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.37 (Me), 46.42 ($CH_2Cl$), 49.07 (*C*HMe), 72.06 (CHOH), 123.50 (*m*-C), 131.72 (*C*C=O), 134.26 (*o*-C) and 168.97 (C=O); m/z (C.I) 254 ($M^+$+1), 271 ($M+NH_4^+$).

**Method B**

***(2S*,3S*)-1-Chloro-2-hydroxy-3-phthaloylaminobutane*** **(403)**

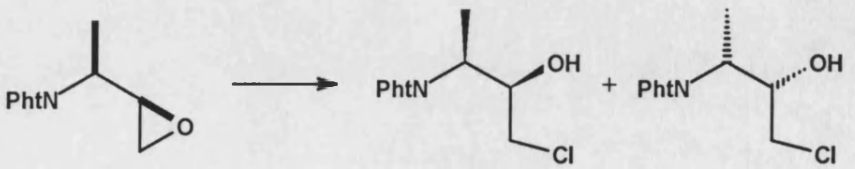
To a stirred solution of epoxide **(323)** (0.386g, 1.78mmol) in THF (10ml) was added lithium chloride (0.45g, 10.66mmol) and acetic acid (0.31ml, 5.33mmol). After stirring the mixture at room temperature for 1 day, the reaction mixture was diluted with EtOAc (20ml), washed with water (3 x 20ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to afford a quantitative yield of chloroalcohol **(403)** (0.453g) as a colourless solid; $R_f$ [petrol:EtOAc (70:30)] 0.55; m.p. 87°C.




**(323)** **(403a)** **(403b)**

***(2R*,3S*)-1-Chloro-2-hydroxy-3-phthaloylaminobutane*** **(404)**

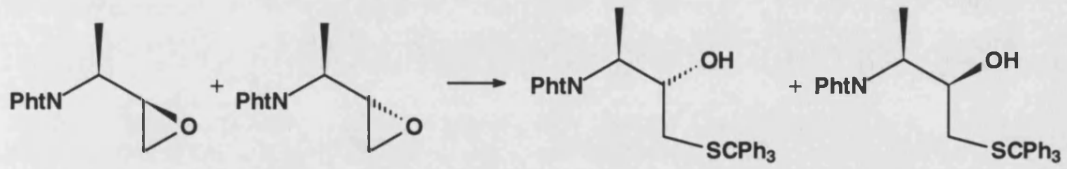
To a stirred solution of epoxide **(322)** (0.514g, 2.37mmol) in THF (10 ml) was added lithium chloride (2.406g, 56.8mmol) and acetic acid (0.38ml, 6.7mmol). After stirring at room temperature for 3 hours, water (30ml) was added and the reaction mixture was then extracted with ether (2 x 30ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to afford the chloroalcohol **(404)** (0.55g, 92%) as a colourless solid; $R_f$ [petrol:EtOAc (70:30)] 0.55; m.p. 119°C.




**(322)** **(404a)** **(404b)**

***(2S,3S) and (2R,3S)-2-Hydroxy-3-phthaloylamino-1-triphenylmethylthiobutane* (409a) *and* (409b)**

To a stirred solution of the racemic epoxides **(322)** and **(323)** (0.143g, 0.66mmol) in anhydrous methanol (6ml) was added triphenylmethylthiol (0.365g, 1.32mmol) and triethylamine (0.275ml, 1.97mmol). The reaction was then stirred for 4 hours at room temperature. After concentrating *in vacuo*, the resulting solid was dissolved in EtOAc (30ml), washed with water (20ml), brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 0.3g of crude material. This was flash chromatographed, eluting with petrol:EtOAc (75:25) to give epoxides (**322:323**, 90:10) (0.014g, 10%) and an inseparable mix of the desired products (**409a:409b**, 38:62) (0.28g, 86%):




**(322)** **(323)** **(409a)** **(409b)**

***(2S,3S)-2-Hydroxy-3-phthaloylamino-1-triphenylmethylthiobutane* (409a)**

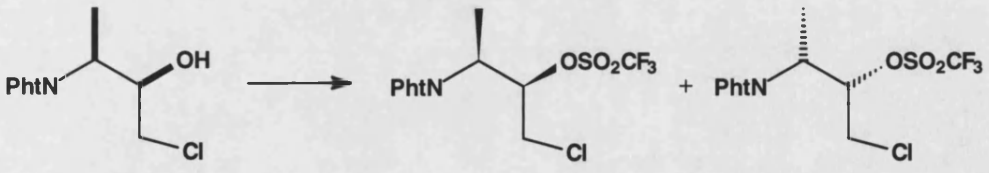
(2*S*,3*S*)-2-Hydroxy-3-phthaloylamino-1-triphenylmethylthiobutane **(409a)** was isolated as a colourless solid; $R_f$[petrol:EtOAc (80:20)] 0.28; $\nu_{max}/cm^{-1}$ $3444_S$ (OH), $1774_S$ (C=O), $1707_s$ (C=O), 1594 and 1026; $\delta_H$(270 MHz, $CDCl_3$) 1.22 (3H, d, *J* 7.2, Me), 2.20 (1H, dd, *J* 12.7 and 5.7, C*H*HS), 2.54 (1H, dd, *J* 12.7 and 7.3, CH*H*S), 3.57 (1H, br s, OH), 3.57-3.63 (1H, m, C*H*OH), 4.27 (1H, qd, *J* 7.0 and 4.0, C*H*Me), 7.00-7.20 (15H, m, $CPh_3$) and 7.70-7.90 (4H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.22 (Me), 36.24 ($CH_2S$), 50.81 (N*C*HMe), 71.84 (CHOH), 123.34 (*m*-C), 131.66 (*C*C=O), 134.1 (*o*-C), 144.52 ($CPh_3$) and 168.53 (C=O); m/z (C.I) 516.3 [5%, ($MNa^+$)], 475 [3%, ($M^+$+1)-$H_2O$)], 218 [100, ($M^+$+1)-$SCPh_3$].

***(2R,3S)-2-Hydroxy-3-phthaloylamino-1-triphenylmethylthiobutane* (409b)**

(2*R*,3*S*)-2-Hydroxy-3-phthaloylamino-1-triphenylmethylthiobutane **(409b)** was isolated as a colourless solid; $R_f$[petrol:EtOAc (80:20)] 0.28; $\nu_{max}/cm^{-1}$ $3444_s$ (OH), $1774_s$ (C=O), $1707_s$ (C=O), 1594 and 1026; $\delta_H$(270 MHz, $CDCl_3$) 1.24 (3H, d, *J* 7.0, Me), 2.23 (1H, dd, *J* 12.7 and 5.7, C*H*HS), 2.45 (1H, dd, *J* 12.5 and 6.8, CH*H*S), 3.57 (1H, d, *J* 8.8, OH), 3.38-3.70 (1H, m, C*H*OH), 4.33 (1H, qd, *J* 6.8 and 6.7, C*H*Me), 7.00-7.20 (15H, m, $CPh_3$) and 7.70-7.90 (4H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.44 (Me), 36.53 ($CH_2S$), 50.74 (N*C*HMe), 71.24 (CHOH), 123.4 (*m*-C), 131.77 (*C*C=O), 134.06 (*o*-C), 144.45 (*C*$Ph_3$) and 169.20 (C=O); m/z (C.I) 516.3 [5%, ($MNa^+$)], 475 [3%, ($M^+$+1)-$H_2O$)], 218 [100, ($M^+$+1)-$SCPh_3$].

***(2R*,3S*)-1-Chloro-3-phthaloylamino-2-(trifluoromethanesulfonyloxy)butane* (463)**

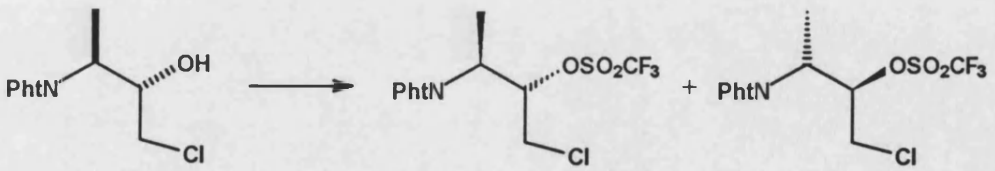
To a cooled solution (-20°C) of the racemic chloroalcohol **(404)** (0.283g, 1.12mmol) in DCM (10ml) was added pyridine (0.09ml, 1.12mmol). The reaction was stirred for 5 minutes before the addition of triflic anhydride (0.226ml, 1.34mmol). It was then allowed to stir at -20°C for 5 minutes before being warmed to room temperature and stirred for a further 1 hour. The reaction mixture was diluted with DCM (10ml), washed with 1M HCl (20ml), brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was used immediately in the next step of the synthesis.




**(404)** **(463a)** **(463b)**

***(2S*,3S*)-1-Chloro-3-phthaloylamino-2-(trifluoromethanesulfonyloxy)butane* (464)**

To a cooled solution (-20°C) of the racemic chloroalcohol **(403)** (0.143g, 0.56mmol) in DCM (3ml) was added pyridine (0.091ml, 1.13mmol). The reaction was stirred for 5 minutes before the addition of triflic anhydride (0.143ml, 0.85mmol). It was then allowed to stir at -20°C for 5 minutes before being warmed to room temperature and stirred for a further 1 hour. The reaction mixture was diluted with DCM (5ml), washed with 1M HCl (5ml), brine (5ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with petrol:EtOAc (70:30) to give the desired product **(464)** (0.156g, 72%) as a colourless solid; m.p. 108-109°C (from ether); (Found C, 40.50; H, 2.82; N, 3.50. calc. for $C_{13}H_{11}NClF_3O_5S$ : C, 40.48; H, 2.87; N, 3.50%); $\nu_{max}/cm^{-1}$ $1772_s$ (C=O), $1708_s$ (C=O) 1607, $1462_{\delta\ as}$ (Me), $1377_{\delta\ s}$ (Me) and 1143; $\delta_H$(270 MHz, $CDCl_3$) 1.61 (3H, d, *J* 7.0, Me), 3.64 (1H, dd, *J* 13.6 and 3.7, C*H*HCl), 3.95 (1H, dd, *J* 13.6 and 3.3, CH*H*Cl), 4.88 (1H, dq, *J* 8.6 and 7.1, CHMe), 5.71 (1H, ddd, *J* 8.6, 3.7 and 3.3, CHO), 7.76-7.80 (2H, m, phthaloyl) and 7.86-7.91 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.84 (Me), 42.7 ($CH_2Cl$), 46.46 (*C*HMe), 86.24 (CHO), 118 (q, $^{C\text{-}F}J$ 309, $CF_3$), 123.67 (*m*-C), 131.44 (*C*C=O), 134.56 (*o*-C) and 167.45 (C=O); m/z (70eV) 281 (3%, M-$CH_2Cl$), 174 (23, M-$F_3CSO_3CHCH_2Cl$); (C.I.) 386 ($M^+$+1, 90).

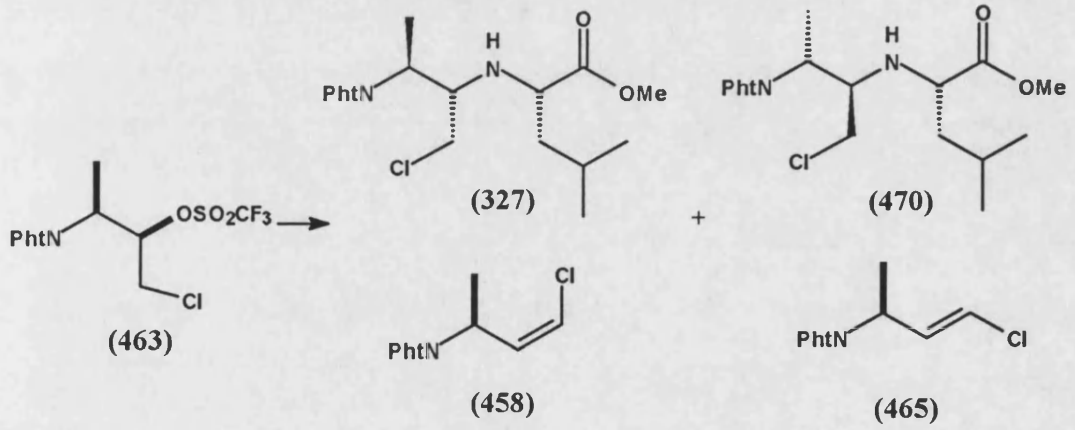



**(403)** **(464a)** **(464b)**

***Preparation of the chloromethyl dipeptides* (326) *and* (327).**

**Method A**

To a stirred solution of the crude racemic chlorotriflate **(463)** (0.426g, 1.12mmol) in THF (20ml) at room temperature was added Leu-OMe.HCl (0.204g, 1.12mmol) and triethylamine (0.167ml, 1.2mmol). The reaction was stirred for one day, after which time the t.l.c. showed no further reaction. The reaction mixture was diluted with EtOAc (10ml), washed with 1M HCl (20ml), brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with petrol:EtOAc:$Et_3N$ (80:20:1) to give an inseparable mixture of the (*Z*) and (*E*)-chloroalkenes (**458:465**, 87:13) (0.031g, 12%). Also isolated was the starting material **(463)** (0.029g, 17%) and an inseparable mix of the chloromethyl dipeptides **(327:470)** (0.145g, 34%).




***(Z)-1-Chloro-3(S*)-phthaloylaminobut-1-ene* (458)**

(*Z*)-1-Chloro-3(*S**)-phthaloylaminobut-1-ene **(458)** was isolated as a pale yellow solid; $R_f$ [petrol: EtOAc (80:20)] 0.9; (Found C, 61.01; H, 4.32; N, 5.8. calc. for $C_{12}H_{10}NO_2Cl$ : C, 61.16; H, 4.28; N, 5.94%); $\nu_{max}/cm^{-1}$ $1766_s$ (C=O), $1719_s$ (C=O), $1705_s$ (C=C), $1464_{\delta\ as}$ (Me), $1377_{\delta\ s}$ (Me), $750_s$ (C-Cl) and 718; $\delta_H$(270 MHz, $CDCl_3$) 1.55 (3H, d, *J* 7.0, Me), 5.44 (1H, dqd, *J* 7.5, 7.0 and 1.2, C*H*Me),

6.16 (1H, dd, *J* 7.5 and 1.2, HC=C*H*Cl), 6.42 (1H, dd, *J* 7.5 and 7.0, *H*C=CHCl) and 7.72-7.88 (4H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 18.70 (Me), 43.30 (*C*HMe), 120.20 (HC=*C*HCl), 123.20 (*m*-C), 130.10 (H*C*=CHCl), 131.90 (*C*C=O), 134.00 (*o*-C) and 167.60 (C=O); m/z (70eV) 220 (15%, M-$CH_3$), 200 (100, M-Cl); (C.I.) 236 ($M^+$+1, 100), 253 (100, M+$NH_4^+$).

***(E)-1-Chloro-3(S*)-phthaloylaminobut-1-ene* (465)**

(*E*)-1-Chloro-3(*S**)-phthaloylaminobut-1-ene **(465)** was isolated as a pale yellow solid, $R_f$ [petrol: EtOAc (80:20)] 0.9; (Found C, 61.01; H, 4.32; N, 5.8. calc. for $C_{12}H_{10}NO_2Cl$ : C, 61.16; H, 4.28; N, 5.94%); $\nu_{max}/cm^{-1}$ $1766_s$ (C=O), $1719_s$ (C=O), $1705_s$ (C=C), $1464_{\delta\ as}$ (Me), $1377_{\delta\ s}$ (Me), $750_s$ (C-Cl) and 718; $\delta_H$(270 MHz, $CDCl_3$) 1.68 (3H, d, *J* 7.0, Me), 5.44 (1H, dqd, *J* 8.0, 7.0 and 1.2, C*H*Me), 6.16 (1H, dd, *J* 8.0 and 1.2, HC=C*H*Cl), 6.34 (1H, dd, *J* 14 and 8.0, *H*C=CHCl) and 7.72-7.88 (4H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 19.0 (Me), 44.0 (*C*HMe), 120.5 (HC=*C*HCl), 123.5 (*m*-C), 130.1 (H*C*=CHCl), 131.9 (*C*C=O), 134.3 (*o*-C) and 167.6 (C=O); m/z (70eV) 220 (15%, M-$CH_3$), 202 (M-Cl, 30), 200 (M-Cl, 100); (C.I.) 236 (100, $M^+$+1), 253 (100, M+$NH_4^+$).

***(2R*,3S*)-1-Chloro-3-phthaloylamino-2-(trifluoromethanesulfonyloxy)butane* (463)**

(2*R**,3*S**)-1-Chloro-3-phthaloylamino-2-(trifluoromethanesulfonyloxy)butane **(463)** was isolated as a colourless solid; m.p. 132°C; (Found C, 40.50; H, 2.82; N, 3.50. calc. for $C_{13}H_{11}NClF_3O_5S$ : C, 40.48; H, 2.87; N, 3.50%); $\nu_{max}/cm^{-1}$ $1772_s$ (C=O), $1708_s$ (C=O), 1607, $1462_{\delta\ as}$ (Me), $1377_{\delta\ s}$ (Me) and 1143; $\delta_H$(270 MHz, $CDCl_3$) 1.60 (3H, d, *J* 7.2, Me), 3.88 (1H, dd, *J* 13.7 and 2.9, C*H*HCl), 4.06 (1H, dd, *J* 13.7 and 2.8, CH*H*Cl), 4.92 (1H, dq, *J* 9.2 and 7.2, C*H*Me), 5.78 (1H, ddd, *J* 9.2, 2.9 and 2.8, CHO), 7.76 (2H, m, phthaloyl) and 7.88 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.30 (Me), 43.46 ($CH_2Cl$), 47.06 (*C*HMe), 84.95 (CHO), 121.9 (q, $^{C\text{-}F}J$ 309, $CF_3$), 123.55 (*m*-C), 131.43 (*C*C=O), 134.41 (*o*-C) and 167.72 (C=O);

m/z (70eV) 281 (3%, M-$CH_2Cl$), 174 (M-$F_3CSO_3CHCH_2Cl$, 23); (C.I.) 386 ($M^+$+1, 90).

***(2S,3S,2'S) and (2R,3R,2'S)-1-Chloro-2-(O-methyl leucinyl)-3-phthaloylamino-butane* (327) *and* (462)**

***(2S,3S,2'S)-1-Chloro-2-(O-methyl leucinyl)-3-phthaloylaminobutane* (327)**

(2*S*,3*S*,2'*S*)-1-Chloro-2-(*O*-methyl leucinyl)-3-phthaloylaminobutane **(327)** as the major isomer, was a colourless gum, $\nu_{max}/cm^{-1}$ $3400_s$ (NH), $1736_s$ (C=O), $1713_s$ (C=O), 1656, $1459_{\delta\ as}$ (Me) and $1370_{\delta\ s}$ (Me); $\delta_H$(270 MHz, $CDCl_3$) 0.92 (3H, d, *J* 6.4, $CH_2$*Me*), 1.05 (3H, d, *J* 6.3, $CH_2$*Me*), 1.41 (3H, d, *J* 7.0, CH*Me*), 1.66-1.80 (1H, m, $CH_2CHMe_2$), 1.80-2.20 (2H, m, $CH_2CHMe_2$), 3.54 (1H, dd, *J* 11.2 and 7.3, CH*H*Cl), 3.65 (1H, dd, *J* 11.2 and 5.7, C*H*HCl), 3.77 (3H, s, OMe), 4.15 (1H, ddd, *J* 7.3, 5.7 and 2.0, CHN), 4.90-5.00 (1H, m, C*H*Me), 5.04-5.12 (1H, m, C*H*$CO_2$Me), 6.00 (1H, br s, NH), 7.29-7.78 (2H, m, phthaloyl), 7.85-8.10 (1H, m, phthaloyl) and 7.90-7.94 (1H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.3 (Me), 21.3 and 22.2 (CH*Me*$_2$), 25.1 (*C*HMe$_2$), 43.4 (*C*$H_2$CHMe$_2$), 45.8 ($CH_2Cl$), 49.5 (N*C*HMe), 52.4 (OMe), 59.0 (N*C*H$CO_2$), 73.6 (CHN), 123.4 (*m*-C), 131.7 (*C*C=O), 134.3 (*o*-C), 166.9 (C=O), 168.3 (C=O) and 172.4 (*C*$O_2$Me); m/z (C.I.) 381 ($M^+$+1, 10%), 345 (5, $M^+$-Cl); [Found : $(M+1)^+$ 381.1587. $C_{19}H_{26}N_2O_4Cl$ requires 381.1581].

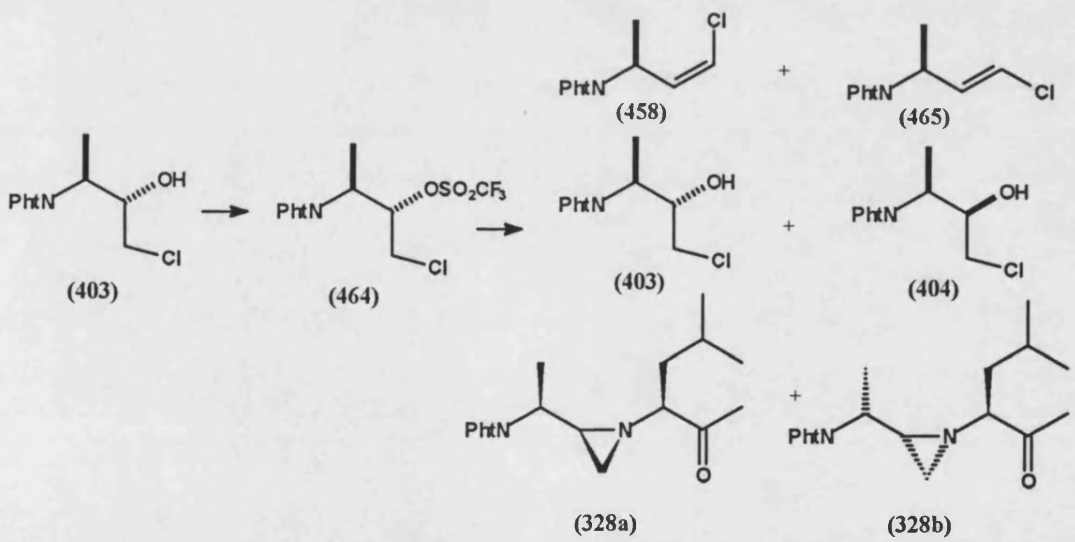
***(2R,3R,2'S)-1-Chloro-2-(O-methyl leucinyl)-3-phthaloylaminobutane* (462)**

(2*R*,3*R*,2'*S*)-1-Chloro-2-(*O*-methyl leucinyl)-3-phthaloylaminobutane **(462)** as the minor isomer, was a colourless gum, $\nu_{max}/cm^{-1}$ $3400_s$ (NH), $1736_s$ (C=O), $1713_s$ (C=O), 1656, $1459_{\delta\ as}$ (Me) and $1370_{\delta\ s}$ (Me); $\delta_H$(270 MHz, $CDCl_3$) the same as ( ) except 0.91 (3H, d, *J* 6.4, $CH_2$*Me*), 1.03 (3H, d, *J* 6.3, $CH_2$*Me*), 1.38 (3H, d, *J* 7.0, CH*Me*) the other peaks were obscured; $\delta_C$ (67.8 MHz, $CDCl_3$) 14.5 (Me), 21.1 and 21.9 (CH*Me*$_2$), 24.8 (*C*HMe$_2$), 43.7 (*C*$H_2$CHMe$_2$), 45.8 ($CH_2Cl$), 49.7

(NCHMe), 53.1 (OMe), 60.2 ($NCHCO_2$), 72.5 (CHN), 124.1 (*m*-C), 131.7 (*C*C=O), 133.7 (*o*-C), 166.9 (C=O), 168.3 (C=O) and 172.4 ($CO_2Me$); m/z (C.I.) 381 ($M^+$+1, 10%), 345 (5, $M^+$-Cl); [Found : $(M+1)^+$ 381.1587. $C_{19}H_{26}N_2O_4Cl$ requires 381.1581].

**Method B**

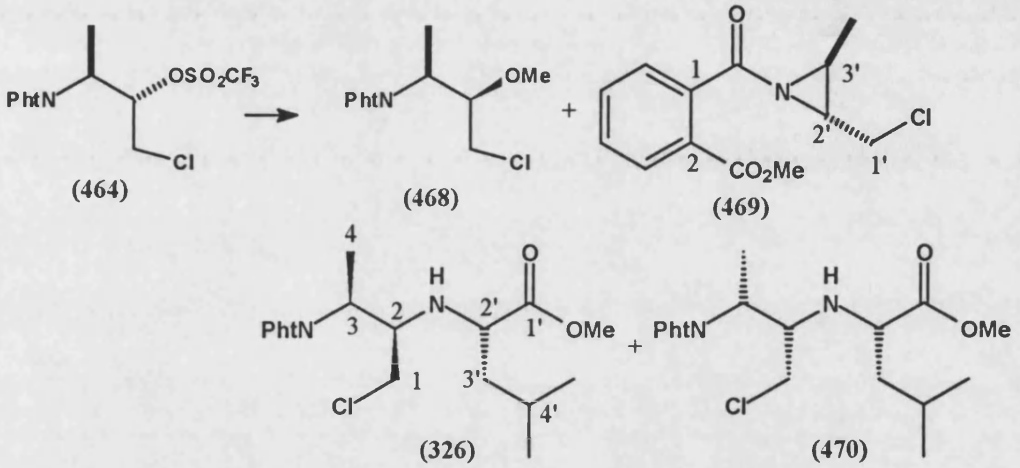
Analogous to method A, the triflate was generated from the racemic chloroalcohol **(403)** (0.108g, 0.43mmol) in DCM (5ml), concentrated *in vacuo* and then reacted immediately with Leu-OMe (1 eq.) and triethylamine (1 eq.). After 3 days more triethylamine (1 eq.) was added and the reaction left for a further 10 days. After chromatography (*Z*) **(458)** and (*E*)-alkene **(465)** (0.005g, 5%), racemic chloroalcohols **(403:404**, ~1:1) (0.063g, 58%) and aziridines **(328)** (0.012g, 8%) were isolated.




**Method C**

To a stirred solution of the racemic chlorotriflate **(464)** (164mg, 0.43mmol) in MeOH (5ml) at room temperature was added Leu-OMe.HCl (86mg, 0.47mmol) and $K_2CO_3$ (0.2g, 0.87mmol). The reaction was stirred for one day, after which

time a t.l.c. showed starting material. So the reaction was heated to 50°C for 3 hours, no further reaction occurred, the reaction mixture was diluted with $CHCl_3$ (10ml), washed with 0.5M HCl (20ml) and the acidic phase back extracted with $CHCl_3$ (10ml). The organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 0.155g of crude material, which was flash chromatographed, eluting with petrol:EtOAc (70:30) gradient to (50:50) to give racemic chloromethyl ether **(472)** (7mg, 6%), chloromethyl dipeptide **(326)** and **(470)** (0.2mg, 0.1%) and racemic chloromethyl aziridine **(469)** (79mg, 69%):




***(2R*,3S*)-1-Chloro-2-methoxy-3-phthaloylaminobutane* (468)**

(2*R**,3*S**)-1-Chloro-2-methoxy-3-phthaloylaminobutane **(468)** was isolated as a colourless oil; $R_f$ [petrol:EtOAc (70:30)] 0.44; $\nu_{max}/cm^{-1}$ $1724_s$ (C=O), 1606, $1464_{\delta\ as}$ (Me), $1356_{\delta\ s}$ (Me), 1318, 1085 and 764; $\delta_H$(270 MHz, $CDCl_3$) 1.54 (3H, d, *J* 6.6, CH*Me*), 3.17 (3H, s, OMe), 3.51 (1H, dd, *J* 11.7 and 5.9, CH*H*Cl), 3.60 (1H, dd, *J* 11.5 and 4.6, C*H*HCl), 4.16 (1H, qd, *J* 6.7 and 5.5, C*H*Me), 4.68 (1H, dd, *J* 10.1 and 5.7, C*H*OMe), 7.55-7.68 (3H, m, phthaloyl) and 7.77-7.82 (1H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 20.63 (Me), 44.18 ($CH_2Cl$), 51.93 (*C*HMe), 53.48 (OMe), 89.73 (*C*HOMe), 124.24 (*m*-C), 132.58 (*C*C=O), 133.50 (*o*-C) and 170.77 (C=O); m/z (70eV) 236 (100%, M-OMe), 218 (M-$CH_2Cl$, 5), 200 (M-HCl

and OMe, 5), 174 (M-MeOCHCH$_2$Cl, 67); (C.I.) 268 (100, M$^+$+1); [Found : (M+1)$^+$ 268.0717. $C_{13}H_{15}NO_3Cl$ requires 268.0740].

***2-Methylcarboxylate-[1'(R*)-chloromethyl-2'(S*)-methylaziridine] benzamide* (469)**

2-Methylcarboxylate-[1'(*R**)-chloromethyl-2'(*S**)-methylaziridine] benzamide **(469)** was isolated as a colourless oil; $R_f$ [petrol:EtOAc (50:50)] 0.44; (Found C, 58.00; H, 5.31; N, 5.23. calc. for $C_{13}H_{14}NO_3Cl$ : C, 58.33; H, 5.27; N, 5.23%); $\nu_{max}$/cm$^{-1}$ $1731_s$ (C=O), $1661_s$ (C=O), 1295 and 1272; $\delta_H$(270 MHz, $CDCl_3$) 1.40 (3H, d, *J* 6.8, Me), 3.65 (1H, dd, *J* 11.4 and 10.3, C*H*HCl), 3.72 (1H, dd, *J* 11.4 and 9.5, CH*H*Cl), 3.89 (3H, s, OMe), 4.17 (1H, dq, *J* 6.4 and 5.7, C*H*Me), 4.40 (1H, dd, *J* 11.7 and 6.1, C*H*NCO), 7.50-7.60 (2H, m, phthaloyl) and 7.70-7.80 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 21.40 (Me), 44.47 (*C*H$_2$Cl), 52.35 (CO$_2$*Me*), 66.18 (*C*HMe), 85.30 (*C*HCH$_2$Cl), 127.88 (*C*CO$_2$Me), 128.95 (*o-C*CON), 129.74 (*o-C*CO$_2$Me), 130.45 (*m-C*CON), 130.99 (*m-C*CO$_2$Me), 131.88 (*C*CON), 162.27 (CON) and 167.64 (*C*O$_2$Me); m/z (70eV) 267 (10%, M$^+$), 252 (M-Me, 10), 236 (M-OMe), 232 (M-Cl, 25), 208 (M-CO$_2$Me, 8); (C.I) 268 (100, M$^+$+1).

**Method D**

To a stirred solution of the racemic chlorotriflate **(464)** (0.22g, 0.57mmol) in DCM (7ml) at room temperature was added Leu-OMe.HCl (0.16g, 0.86mmol) and $K_2CO_3$ (0.16g, 1.14mmol). The reaction was stirred for four days, after which time a t.l.c. showed no starting material. The reaction mixture was diluted with $CHCl_3$ (10ml), washed with 0.5M HCl (20ml) and the acidic phase back extracted with $CHCl_3$ (10ml). The organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 0.33g of a crude material. This was flash chromatographed, eluting with petrol:EtOAc (70:30) gradient to (50:50) to give racemic chlorotriflate (464) (0.05g, 23%); $R_f$ [petrol: EtOAc (80:20)] 0.33; and the chloroalcohols; $R_f$ [petrol: EtOAc (80:20)] 0.19-0.16 (403) and (404) and the chloromethyl dipeptides **(326)**

and **(470)**, $R_f$ [petrol:EtOAc (80:20)] 0.14. The mixture was chromatographed several times, eluting with petrol:EtOAc (80:20) until a sample was pure enough for characterisation, chloromethyl dipeptides **(326)** and **(470)** (0.04g, 20%):

***(2R,3S,2'S) and (2S,3R,2'S)-1-Chloro-2-(O-methyl leucinyl)-3-phthaloylamino-butane* (326) *and* (470)**

were isolated as a colourless gum, $\nu_{max}/cm^{-1}$ $3400_s$ (NH), $1736_s$ (C=O), $1713_s$ (C=O), $1656_s$ (C=O), $1459_{\delta\ as}$ (Me) and $1370_{\delta\ s}$ (Me); $\delta_H$(270 MHz, $CDCl_3$) major isomer (2*R*, 3*S*, 2'*S*) (326) 0.85 (3H, d, *J* 6.4, $CH_2$*Me*), 0.97 (3H, d, *J* 6.3, $CH_2$*Me*), 1.40 (3H, d, *J* 7.3, CH*Me*), 1.60-1.66 (1H, m, $CH_2CHMe_2$), 1.68-2.00 (2H, m, $CH_2CHMe_2$), 3.40-3.60 (2H, m, $CH_2Cl$), 3.70 (3H, s, OMe), 4.14-4.22 (1H, m, CHN), 4.94-5.10 (2H, m, C*H*Me and $CHCO_2Me$), 6.00 (1H, br s, NH), 7.60-7.70 (2H, m, phthaloyl) and 7.78-7.90 (1H, m, phthaloyl) and minor isomer (2*S*, 3*R*, 2'*S*) (470) the same as the major except 0.86 (3H, d, *J* 6.4, $CH_2$*Me*), 0.96 (3H, d, *J* 6.3, $CH_2$*Me*), 1.42 (3H, d, *J* 7.3, CH*Me*) all other peaks obscured; $\delta_C$ (67.8 MHz, $CDCl_3$) 14.08 (Me), 21.01 and 22.10 ($CHMe_2$), 25.15 ($CHMe_2$), 43.37 ($CH_2Cl$), 45.52 ($CH_2CHMe_2$), 49.17 (NCHMe), 52.48 (OMe), 60.36 ($NCHCO_2$), 73.19 (CHN), 123.51 (*m*-C), 125.23 (*m*-C), 129.16 (*C*C=O), 132.69 (*o*-C), 133.62 (*o*-C), 168 (C=O) and 172 ($CO_2Me$); m/z (C.I.) 381 ($M^+$+1, 10%), 345 (5, $M^+$-Cl); [Found : $(M+1)^+$ 381.1587. $C_{19}H_{26}N_2O_4Cl$ requires 381.1581].

**Method E**

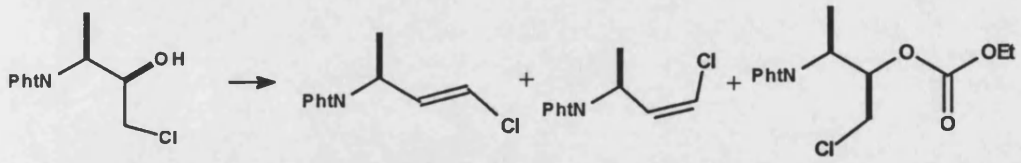
To a stirred solution of the racemic chlorotriflate **(464)** (0.223g, 0.58mmol) in DCM (2ml) at room temperature was added Leu-OMe.HCl (0.136g, 0.75mmol) and DIPEA (0.101g, 0.58mmol). The reaction was stirred for 16 hours, after which time the reaction mixture was refluxed for 2 hours using THF as a co-solvent. After this time a t.l.c. showed no starting material and the reaction mixture was diluted with ether (30ml), washed with 0.5M HCl (50ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 0.265g crude material. This was flash

chromatographed, eluting with petrol:EtOAc (70:30) to give chlorotriflate **(464)** (0.05g, 23%); $R_f$ [petrol:EtOAc (80:20)] 0.33, and the chloroalcohols **(403)** and **(404)**; $R_f$ [petrol:EtOAc (80:20)] 0.19-016 and the chloromethyl dipeptides **(326)** and **(470)** (0.182g, 24%); $R_f$ [petrol:EtOAc (80:20)] 0.14. The mixture was rechromatographed, eluting with petrol:EtOAc (80:20).

***Attempted preparation of the chloromethyl dipeptide* (327) *using the Mitsunobu reaction***

**Method A**

To a solution of the racemic chloroalcohol **(404)** (0.0875g, 0.345mmol), triphenyl phosphine (0.136g, 0.863mmol) and Leu-OMe (0.06g, 0.41mmol) in THF (5ml) was added dropwise DEAD (0.136ml, 0.863mmol). The reaction was stirred at room temperature for 2 days. After this time no new products were observed. The reaction was concentrated *in vacuo* to give 0.53g of crude material which was flash chromatographed on silica, eluting with petrol:EtOAc (90:10 with a gradient to 50:50) to give racemic (*Z*)- **(458)** and (*E*)-alkenes **(465)** (0.046g, 57%), racemic ethyl-carbonate **(459)** (0.009g, 8%) and racemic chloroalcohol **(404)** (0.023g, 26%).




**(404)** **(465)** **(458)** **(459)**

***(2R*,3S*)-1-Chloro-2-ethylcarbonate-3-phthaloylaminobutane* (459)**

(2*R**,3*S**)-1-Chloro-2-ethylcarbonate-3-phthaloylaminobutane **(459)** was isolated as a colourless oil; $\nu_{max}/cm^{-1}$ 2924, 2853, $1774_s$ (C=O), $1747_s$ (C=O), $1713_s$ (C=O), 1612, $1467_{\delta\ as}$ (Me), $1381_{\delta\ s}$ (Me), 1255 and $722_s$ (C-Cl); $\delta_H$(270 MHz, $CDCl_3$) 1.13 (3H, t, *J* 6.9, $CH_2CH_3$), 1.56 (3H, d, *J* 7.2, CH*Me*), 3.76 (1H,dd, *J*

12.5 and 4.1, C*H*HCl), 3.99 (1H, dd, *J* 12.5 and 3.1, CH*H*Cl), 4.01-4.11 (2H, m, $OCH_2CH_3$), 4.78 (1H, qd, *J* 9.1 and 7.2, C*H*Me), 5.54-5.62 (1H, m, CHO), 7.67-7.73 (2H, m, phthaloyl) and 7.81-7.89 (2H, m, phthaloyl); m/z (FAB +ve) 326 ($M^+$+1).

**Method B**

To a solution of the racemic chloroalcohol **(463)** (0.126g, 0.5mmol), triphenyl phosphine (0.197g, 0.75mmol) and Boc-Leu-OMe (0.123g, 0.75mmol) in THF (3ml) was added dropwise DEAD (0.086ml, 0.75mmol). The reaction was stirred at room temperature for 2 days. After this time no new products were observed. The reaction was concentrated *in vacuo* to give 0.56g of a crude material which was flash chromatographed on silica, eluting with petrol:EtOAc (80:20 with a gradient to 60:40) to give Boc-Leu-OMe and racemic (*Z*) **(458)** and (*E*)-alkenes **(465)** (0.104g), racemic carbonate **(459)** (0.002g, 2%) and racemic chloroalcohol **(463)** (0.072g, 57%). A sample (0.02g) of the chloroalcohol **(463)** and ethylcarbonate **(459)** were further purified by HPLC using an isocratic eluent (acetonitrile:water):water:TFA (30:70:1) then after 15 mins gradient to 80:20:1. **Table 30** shows the compounds isolated.

**Table 30**

| Time (minutes) | Area (%) | Compound | Yield, (mg) |
|---|---|---|---|
| 14.3 | 39.3 | chloroalcohol **(463)** | 5 |
| 15.3 | 26.5 | **(463)** + **(393)** | 6 |
| 16.4 | 21.6 | **(463)** + **(393)** | 1 |
| 23.8 | 7.0 | carbonate **(459)** | 1 |

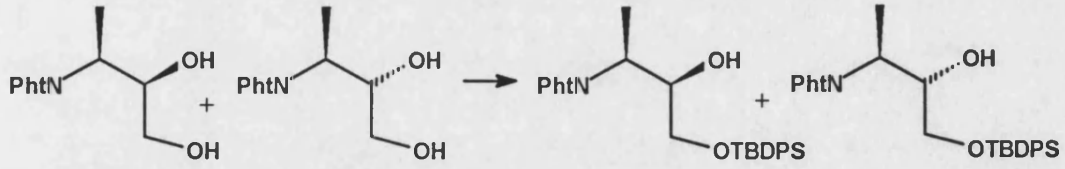

(404) (393)

**Method C**

To a solution of the racemic chloroalcohol **(404)** (0.042g, 0.165mmol), triphenyl phosphine (0.043g, 0.165mmol) and TFA-Leu-OMe (0.04g, 0.165mmol) in THF (5ml) was added dropwise DEAD (0.026ml, 0.165mmol). The reaction was stirred at room temperature for 2 days, after which time no new products were observed. The reaction was concentrated *in vacuo* to give 0.56g of crude material which was flash chromatographed on silica, eluting with petrol:EtOAc (80:20 with a gradient to 60:40) to give recovered TFA-Leu-OMe (0.031g, 77%) and racemic chloroalcohol **(404)** (0.037g, 88%).

***(2S,3S) and (2R,3S)-1-(tert-Butyldiphenylsiloxy)-2-hydroxy-3-phthaloylamino-butane* (339) *and* (474)**

To a solution of the diols **(337)** and **(338)** (0.562g, 2.4mmol) in anhydrous DCM (25ml) was added imidazole (0.409g, 6.0mmol) and *t*-butylchlorodiphenylsilane (0.657g, 2.45mmol). The reaction was stirred under $N_2$ at room temperature for 30 minutes. The reaction was diluted with ether (50ml) and washed with 0.5M HCl (50ml), brine (50ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to give 1.21g of crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to afford the mono protected diols **(474)** and **(339)** (37:63), (1.112g, 98%).




**(337)** **(338)** **(339)** **(474)**

***(2R,3S)-1-(tert-Butyldiphenylsiloxy)-2-hydroxy-3-phthaloylaminobutane* (339)**

(2*R*,3*S*)-1-(*tert*-Butyldiphenylsiloxy)-2-hydroxy-3-phthaloylaminobutane **(339)** was isolated as a colourless solid, $R_f$ [petrol:EtOAc (85:15)]; 0.24; m.p. 97°C (hexane); $[\alpha]_{589}^{22}$0.9 (*c* 0.424 in $CHCl_3$); (Found C, 70.97; H, 6.64; N, 2.69. calc. for $C_{12}H_{13}NO_4$ : C, 71.0; H, 6.60; N, 2.96%); $\nu_{max}/cm^{-1}$ $3453_s$ (OH), $1781_s$ (C=O), $1705_s$ (C=O), 1393, $1108_s$ (Si-C or C-O) and 699; $\delta_H$(270 MHz, $CDCl_3$) 1.05 (9H, s, *t*-Bu), 1.36 (3H, d, *J* 7.2, Me), 3.20 (1H, br d, *J* 7.9, CHO*H*), 3.69 (2H, d, *J* 4.5, $CH_2O$), 4.10 (1H, m, CHO), 4.64 (1H, dq, *J* 7.2 and 7.2, CHMe), 7.2-7.48 (5H, m, ph), 7.58-7.66 (5H, m, ph), 7.70-7.74 (2H, m, phthaloyl) and 7.81-7.85 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.15 (Me), 19.12 (*C*$Me_3$), 26.76 (C*Me*$_3$), 48.78 (N*C*HMe), 64.78 ($CH_2O$), 72.28 (CHOH), 123.21 (*m*-C), 127.67 (Si-Ph), 127.74 (Si-Ph), 129.77 (Si-Ph), 129.83 (Si-Ph), 131.94 (*C*C=O), 132.78 (Si-Ph), 133.91 (*o*-C), 135.47 (Si-Ph), 135.56 (Si-Ph) and 168.95 (C=O); m/z (C.I.) 474 ($M^+$+1, 100%).

***(2S,3S)-1-(tert-Butyldiphenylsiloxy)-2-hydroxy-3-phthaloylaminobutane* (474)**

(2*S*,3*S*)-1-(*tert*-Butyldiphenylsiloxy)-2-hydroxy-3-phthaloylaminobutane **(474)** was isolated as a colourless solid; $R_f$ [petrol:EtOAc (85:15)]; 0.29; m.p. 90-91°C (hexane); $[\alpha]_{589}^{22}$-8.3 (*c* 0.65 in $CHCl_3$); (Found C, 70.97; H, 6.64; N, 2.69. calc. for $C_{12}H_{13}NO_4$ : C, 71.00; H, 6.60; N, 2.96%); $\nu_{max}/cm^{-1}$ $3453_s$ (OH), $1781_s$ (C=O), $1705_s$ (C=O), 1393, $1108_s$ (Si-C or C-O) and 699; $\delta_H$(270 MHz, $CDCl_3$) 1.07 (9H, s, *t*-Bu), 1.37 (3H, d, *J* 7.0, Me), 3.59 (1H, dd, *J* 10.5 and 6.4, C*H*HO), 3.64 (1H, br s, CHO*H*), 3.69 (1H, dd, *J* 10.4 and 5.0, CH*H*O), 4.17 (1H, m, CHO), 4.65 (1H, qd, *J* 7.0 and 5.0, CHMe), 7.25-7.40 (5H, m, ph), 7.59-7.69 (5H, m, ph), 7.70-7.73

(2H, m, phthaloyl) and 7.81-7.84 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 13.05 (Me), 19.12 (*C*$Me_3$), 26.75 (C*Me*$_3$), 48.38 (N*C*HMe), 64.25 ($CH_2O$), 72.96 (CHOH), 123.38 (*m*-C), 127.62 (Si-Ph), 127.77 (Si-Ph), 129.69 (Si-Ph), 129.83 (Si-Ph), 131.79 (*C*C=O), 132.91 (Si-Ph), 134.04 (*o*-C), 135.47 (Si-Ph), 135.56 (Si-Ph) and 168.66 (C=O); m/z (C.I.) 474 ($M^+$+1, 100%).

***(2R,3S)-1-(tert-Butyldiphenylsiloxy)-3-phthaloylamino-2-(trifluoromethane-sulfonyloxy)butane* (340)**

To a cooled solution (-20°C) of the protected diol **(339)** (0.063g, 0.132mmol) in DCM (3ml) was added pyridine (0.012ml, 0.152mmol). The reaction was stirred for 5 minutes before the addition of triflic anhydride (0.024ml, 0.145mmol). The reaction was then stirred at -20°C for 5 minutes before being allowed to attain room temperature at which it was stirred for a further 1 hour. The reaction mixture was diluted with ether (5ml), washed with water (5ml), brine (5ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with petrol:EtOAc (90:10) to give the desired product **(340)** (0.071g, 89%); as a pale pink solid, m.p. 93°C; (Found C, 57.60; H, 4.91; N, 2.28. calc. for $C_{29}H_{30}NO_6F_3SiS$ : C, 57.50; H, 4.99; N, 2.31%); $[\alpha]_{589}^{17}$ 17.5 (*c* 1.4 in $CHCl_3$); $\nu_{max}/cm^{-1}$ $1770_s$ (C=O), $1716_s$ (C=O), 1612, 1590, $1470_{\delta\ as}$ (Me), 1411 and 1388; $\delta_H$(270 MHz, $CDCl_3$) 1.13 (9H, s, *t*-Bu), 1.51 (3H, d, *J* 7.2, Me), 3.96 (1H, dd, *J* 13 and 3.1, C*H*HO), 4.06 (1H, dd, *J* 13 and 2, CH*H*O), 5.02 (1H, dq, *J* 9.7 and 7.2, C*H*Me), 5.53 (1H, ddd, *J* 9.7, 3.1 and 2, CHO) 7.40-7.50 (5H, m, ph), 7.67-7.72 (5H, m, ph), 7.72-7.76 (2H, m, phthaloyl) and 7.85-7.9 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.58 (Me), 19.27 (*C*$Me_3$), 26.67 (C*Me*$_3$), 46.39 (N*C*HMe), 63.01 ($CH_2O$), 88.72 (CHOH), 123.38 (*m*-C), 127.71 (Si-Ph), 127.77 (Si-Ph), 127.99 (Si-Ph), 129.80 (Si-Ph), 129.93 (Si-Ph), 130.13 (Si-Ph), 131.66 (*C*C=O), 131.79 (Si-Ph), 134.22 (*o*-C), 135.52 (Si-Ph), 135.61 (Si-Ph) and 167.86 (C=O); m/z (C.I.) 606 ($M^+$+1, 100%).


**(339)** **(340)**

***(2S,3S)-1-(tert-Butyldiphenylsiloxy)-3-phthaloylamino-2-(trifluoromethane-sulfonyloxy)butane* (475)**

To a cooled solution (-20°C) of the protected diol **(474)** (0.822g, 1.74mmol) in DCM (20ml) was added pyridine (0.16ml, 1.91mmol). The reaction was stirred for 5 minutes before the addition of triflic anhydride (0.35ml, 2.08mmol). The reaction was then stirred at -20°C for 5 minutes before being allowed to attain room temperature at which it was stirred for a further 1 hour. The reaction mixture was diluted with ether (30ml), washed with water (30ml), brine (30ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with petrol:EtOAc (90:10) to give the desired product **(475)** (0.378g, 36%) as a syrup, $R_f$ [petrol:EtOAc (75:25)]; 0.38; $[\alpha]_{589}^{17}$ 0.0 (*c* 0.976 in $CHCl_3$); $\nu_{max}/cm^{-1}$ $1777_s$ (C=O), $1716_s$ (C=O), 1597, $1464_{as}$ (Me) and 1388; $\delta_H$(270 MHz, $CDCl_3$) 0.96 (9H, s, *t*-Bu), 1.49 (3H, d, *J* 7.2, Me), 3.64 (1H, dd, *J* 12.8 and 3.9, C*H*HO), 3.80 (1H, dd, *J* 12.8 and 2.9, CH*H*O), 4.84 (1H, dq, *J* 9.0 and 7.2, C*H*Me), 5.50 (1H, ddd, *J* 9.0, 3.9 and 2.9, CHO) 7.23-7.50 (10H, m, ph) and 7.60-7.80 (4H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.1 (Me), 19.0 (*C*$Me_3$), 26.5 (C*Me*$_3$), 45.6 (NCHMe), 62.6 ($CH_2O$), 88.9 (CHOH), 123.4 (*m*-C), 127.6 (Si-Ph), 127.7 (Si-Ph), 127.9 (Si-Ph), 129.7 (Si-Ph), 129.9 (Si-Ph), 130.1 (Si-Ph), 131.6 (*C*C=O), 131.8 (Si-Ph), 134.3 (*o*-C), 135.3 (Si-Ph), 135.5 (Si-Ph) and 167.4 (C=O); m/z (C.I.) 606 ($M^+$+1, 100%).


**(474)** **(475)**

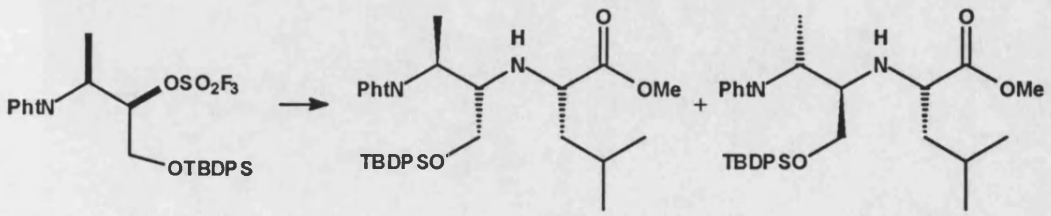
***Attempted preparation of the tert-butyldiphenylsiloxymethyl dipeptide* (476) *using the Mitsunobu reaction***

To a solution of the protected diol **(339)** (0.159g, 0.336mmol), triphenyl phosphine (0.097g, 0.37mmol) and TFA-Leu-OMe **(368)** (0.085g, 0.336) in THF (3ml) was added dropwise DEAD (0.058ml, 0.37mmol). The reaction was stirred at room temperature for 4 days after which time no new products were observed. The reaction was concentrated *in vacuo* and flash chromatographed, eluting with cyclohexane:EtOAc (70:30) to give starting materials.

***Preparation of (2S,3S,2'S)-1-(tert-butyldiphenylsiloxy)-2-(O-methyl leucinyl)-3-phthaloylaminobutane* (476) *by displacement of the TBDPS protected triflate* (340).**

**Method A**

To a stirred a solution of racemic triflate **(340)** (0.08g, 0.132 mmol) in anhydrous DCM (2 ml) was added Leu-OMe.HCl (0.03g, 0.16mmol) and triethylamine (0.017ml, 0.16mmol). The reaction was stirred at room temperature for 3 days before being diluted with ether (20 ml). The organics were washed with water (30 ml), dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) gradient petrol:EtOAc (80:20). This gave the starting material **(340)** (0.041g, 51%) and an intractable mix of the hydroxymethyl dipeptides **(476:477)** (0.033g, 40%):




**(340)** **(476)** **(477)**

***(2S,3S,2'S)-1-(tert-Butyldiphenylsiloxy)-2-(O-methyl leucinyl)-3-phthaloylamino butane* (476).**

(2*S*,3*S*,2'*S*)-1-(*tert*-Butyldiphenylsiloxy)-2-(*O*-methyl leucinyl)-3-phthaloylamino-butane (**476**) was isolated as a colourless oil; $R_f$ [petrol:ether(60:40)] 0.27; (Found C, 70.5; H, 7.39; N, 4.22. calc. for $C_{35}H_{44}N_2O_5Si$ : C, 69.97; H, 7.38; N, 4.66%); $\nu_{max}/cm^{-1}$ $3388_s$ (NH), $1735_s$ (C=O), $1713_s$ (C=O), 1656, $1470_{\delta\ as}$ (Me), 1430 and 1387; $\delta_H$(400 MHz, $CDCl_3$) 0.91 (3H, d, *J* 6.4, CH*Me*$_2$), 1.03 (3H, d, *J* 6.4, CH*Me*$_2$), 1.11 (9H, s, $^t$Bu), 1.23 (3H, d, *J* 7.0, CH*Me*), 1.68-1.76 (1H, m, $CH_2CHMe_2$), 1.86-1.98 (2H, m, $CH_2CHMe_2$), 3.50-3.60 (1H, m, CH*H*O) 3.72 (3H, s, OMe), 3.84 (1H, ddd, *J* 10.3, 4.8 and 2.4, CHN), 4.01-4.17 (1H, m, C*H*HO), 5.02-5.12 (1H, m, $CHCO_2Me$), 5.20 (1H, brq, *J* 7.3, C*H*Me), 5.80-6.00 (1H, brs, NH), 7.22-7.34 (5H, m, phenyl); 7.58-7.65 (7H, m, phenyl and phthaloyl) and 7.82-7.88 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 11.02 (CH*Me*), 19.17 ($CMe_3$), 21.92 (CH*Me*$_2$), 22.85 (CH*Me*$_2$), 25.06 ($CHMe_2$), 26.67 (C*Me*$_3$), 26.78 (C*Me*$_3$), 26.84 (C*Me*$_3$), 43.75 ($CH_2$), 48.82 (*C*HMe), 52.44 (OMe), 60.41 ($CHCO_2$), 64.72 ($CH_2O$), 73.73 (CHN), 123.25 (SiPh), 124.03 (SiPh), 125.32 (*m*-C), 127.6 (SiPh), 129.4 (*C*C=O), 129.56 (SiPh), 129.76 (SiPh), 132.38 (SiPh), 132.91 (SiPh), 133.29 (SiPh), 133.95 (*o*-C), 135.59 (SiPh), 167.50 (CON), 168.00 (CON) and 172.00 ($CO_2Me$); m/z (C.I.) 601 ($M^+$+1, 100%).

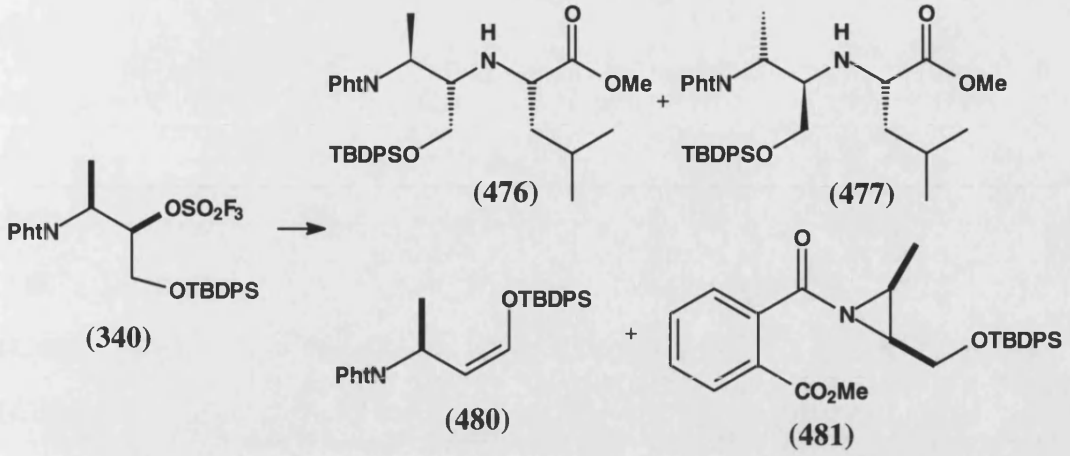
***(2R,3R,2'S)-1-(tert-Butyldiphenylsiloxy)-2-(O-methyl leucinyl)-3-phthaloyl-amino butane* (477).**

(2*R*,3*R*,2'*S*)-1-(*tert*-Butyldiphenylsiloxy)-2-(*O*-methyl leucinyl)-3-phthaloylamino butane (**477**) was isolated as a colourless gum; $R_f$ [petrol:ether(60:40)] 0.27; (Found C, 70.5; H, 7.39; N, 4.22. calc. for $C_{35}H_{44}N_2O_5Si$ : C, 69.97; H, 7.38; N, 4.66%); $\nu_{max}/cm^{-1}$ $3388_s$ (NH), $1735_s$ (C=O), $1713_s$ (C=O), 1656, $1470_{\delta\ as}$ (Me), 1430 and 1387; $\delta_H$(270 MHz, $CDCl_3$) 0.85-1.06 (6H, m, CH*Me*$_2$), 1.10 (9H, s, $^t$Bu), 1.22 (3H, m, CH*Me*), 1.68-1.76 (1H, m, $CH_2CHMe_2$), 1.86-1.98 (2H, m, $CH_2CHMe_2$), 3.71 and 3.73 (3H, s, OMe), 3.79-3.98 (1H, m, CHN), 4.01-4.17

(2H, m, $CH_2O$), 4.96-5.05 (1H, m, C*H*$CO_2$Me), 5.20 (1H, m, C*H*Me), 7.22-7.34 (5H, m, phenyl); 7.58-7.65 (7H, m, phenyl and phthaloyl) and 7.82-7.88 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 10.78 (CH*Me*), 19.17 (C*Me*$_3$), 21.12 (CH*Me*$_2$), 22.78 (CH*Me*$_2$), 25.06 (*C*HMe$_2$), 26.67 (C*Me*$_3$), 26.78 (C*Me*$_3$), 26.84 (C*Me*$_3$), 43.46 ($CH_2$), 48.73 (*C*HMe), 52.37 (OMe), 60.27 (*C*H$CO_2$), 64.72 ($CH_2O$), 73.53 (CHN), 123.32 (SiPh), 124.07 (SiPh), 125.32 (*m*-C), 127.68 (SiPh), 129.40 (*C*C=O), 129.65 (SiPh), 129.87 (SiPh), 132.38 (SiPh), 132.91 (SiPh), 133.29 (SiPh), 134.08 (*o*-C), 135.7 (SiPh), 167.50 (CON), 168.00 (*C*ON) and 172.00 (*C*$O_2$Me); m/z (C.I.) 601 ($M^+$+1, 100%).

**Method B**

To a stirred a solution of racemic triflate **(340)** (0.89g, 1.47mmol) in anhydrous MeOH (15 ml) was added Leu-OMe.HCl (0.294g, 1.62mmol) and $K_2CO_3$ (0.406g, 2.94mmol). The reaction was stirred at room temperature for 1 day before being diluted with $CHCl_3$ (50ml). The organics were washed with 0.5M HCl (30 ml), back extracted with $CHCl_3$ (60ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to give 0.812g of crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to give the enol ether **(480)** (0.01g, 1.5%), hydroxymethyl dipeptides **(476)** and **(477)** (0.134g, 15%) and the aziridine **(481)** (0.468g, 65%):




***(Z)-1-(tert-Butyldiphenylsilyloxy)-3(S*)-phthaloylaminobut-1-ene*** **(480)**

(*Z*)-1-(*tert*-Butyldiphenylsilyloxy)-3(*S**)-phthaloylaminobut-1-ene **(480)** was isolated as a colourless oil; $R_f$ [petrol:ether (60:40)] 0.35; (Found C, 73.5; H, 6.99; N, 2.61. calc. for $C_{28}H_{29}NO_3Si$ : C, 73.8; H, 6.42; N, 3.07%); $\nu_{max}/cm^{-1}$ $1774_s$ (C=O), $1712_s$ (C=O), $1654_s$ (C=C), $1468_{\delta\ as}$ (Me), and $1116_s$ (Si-C); $\delta_H$(270 MHz, $CDCl_3$) 1.06 (9H, s, *t*-Bu), 1.55 (3H, d, *J* 7.2, Me), 5.11 (1H, dd, *J* 8.4 and 5.7, C=C*H*CHMe), 5.70 (1H, dq, *J* 8.3 and 7.2, C*H*Me), 6.17 (1H, dd, *J* 5.7 and 1.1, *H*COTBDPSi), 7.20-7.50 (6H, m, SiPh), 7.55-7.6 (2H, m, SiPh), 7.64-7.74 (4H, m, phthaloyl and SiPh) and 7.80-7.84 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.10 (Me), 26.50 (*C*$Me_3$), 26.60 (C*Me*$_3$), 41.24 (N*C*HMe), 109.16 (C=*C*HCHMe), 122.96 (*m*-C), 127.63 (Si-Ph), 127.68 (Si-Ph), 127.73 (Si-Ph), 127.84 (Si-Ph), 129.60 (Si-Ph), 129.93 (Si-Ph), 130.05 (Si-Ph), 132.32 (*C*C=O), 133.62 (Si-Ph), 134.80 (*o*-C), 135.32 (Si-Ph), 135.42 (Si-Ph), 140.50 (*C*=CHCHMe) and 167.93 (C=O); m/z (C.I.) 456 ($M^+$+1, 100%).

***2-Methylcarboxylate-[1'(S*)-1-(tert-butyldiphenylsilyloxy)methyl-2'(S*)-methyl-aziridine] benzamide*** **(481)**

2-Methylcarboxylate-[1'(*S**)-1-(*tert*-butyldiphenylsilyloxy)methyl-2'(*S**)-methyl-aziridine] benzamide **(481)** was isolated as a colourless solid; m.p. 57°C; $R_f$ [petrol:ether (60:40)] 0.3; $\nu_{max}/cm^{-1}$ $1730_s$ (C=O), $1646_s$ (C=O), 1591, $1292_s$ (C-O-

C), 1256 and $1109_s$ (Si-O); $\delta_H$(270 MHz, $CDCl_3$) 1.06 (9H, s, *t*-Bu), 1.30 (3H, d, *J* 7.0, Me), 3.77 (3H, s, OMe), 3.90 (2H, dd, *J* 6.2 and 1.6, C$H_2$OTBDPSi), 4.45 (1H, dq, *J* 9.0 and 7.0, C*H*Me), 4.75 (1H, d, *J* 9.0 and 6.2, NC*H*), 7.35-7.44 (6H, m, SiPh), 7.46-7.52 (2H, m, phthaloyl), 7.64-7.74 (6H, m, phthaloyl and SiPh) and 7.80-7.84 (6H, m, SiPh and phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 15.32 (Me), 26.69 (*C*$Me_3$), 26.73 (C*Me*$_3$), 52.32 ($CO_2$*Me*), 61.97 ($CH_2O$), 63.22 (N*C*HMe), 82.56 (*C*H$CH_2O$), 127.68 (Si-Ph), 127.76 (Si-Ph), 128.02 (*C*$CO_2$Me), 128.75 (*o*-*C*CON), 129.68 (Si-Ph), 129.74 (*o*-*C*$CO_2$Me), 130.32 (*m*-*C*CON), 132.29 (*m*-*C*$CO_2$Me), 133.08 (Si-Ph), 133.14 (*C*CON), 135.51 (Si-Ph), 162.30 (NC=O) and 167.70 (*C*$O_2$Me); m/z (70eV) 430 (5%, M-$^t$Bu), 269 (8, $CH_2$OSiTBDP$^+$); (C.I.) 488 ($M^+$+1, 100%); [Found : $(M+1)^+$ 488.2254. $C_{29}H_{34}NO_4Si$ requires 488.2257].

**Method C**

To a stirred a solution of racemic triflate **(340)** (0.56g, 0.925mmol) in anhydrous DCM (5 ml) was added Leu-OMe.HCl (0.202g, 1.11mmol), triethylamine (0.36ml, 2.6mmol) and catalytic amount of DMAP (0.005g, 0.05mmol). The reaction was allowed to stir at room temperature for 2 days before refluxing for 3 hours. The reaction mixture was then diluted with ether (10ml), the organics washed with 0.5M HCl (20 ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to give 0.83g of crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to give the racemic enol ether **(480)** (0.06g, 12%) and the hydroxymethyl dipeptides **(476)** and **(477)** (0.111g, 20%).

**Method D**

To a stirred a solution of the racemic triflate **(340)** (0.23g, 0.38mmol) in anhydrous THF (4ml) was added Leu-OMe.HCl (0.083g, 0.46mmol) and DIPEA (0.146ml, 0.84mmol). The reaction was allowed to stir at room temperature for 2 days before refluxing for 3 hours. The reaction mixture was then diluted with ether (10ml), the organics were washed with 0.5M HCl (20 ml), dried ($Na_2SO_4$) and concentrated *in*

*vacuo*.. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to give starting material (0.12g, 52%) and the hydroxymethyl dipeptides (**476**) and (**477**) (0.07g, 31%).

**Method E**

To a stirred a solution of racemic triflate (**340**) (0.523g, 0.86mmol) in anhydrous DCM (2 ml) was added Leu-OMe.HCl (0.164g, 0.9mmol) and DIPEA (0.301ml, 1.73mmol). The reaction was allowed to stir at room temperature for 1 day before refluxing for 12 hours. The reaction mixture was then diluted with ether (10ml), the organics were washed with 0.5M HCl (20 ml), dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with gradient petrol:EtOAc (90:10) to (85:15) to give starting material (**340**) (0.015g, 3%), racemic enol ether (**480**) (0.08g, 20%) and the hydroxymethyl dipeptides (**476**) and (**477**) (0.278g, 56%).

***(2S,3S)-1-(tert-Butyldiphenylsilyloxy)-2-(O-methyl leucinyl)-3-phthaloylamino-butane* (476).**

To a stirred a solution of the triflate (**340**) (2.39g, 3.95mmol) in anhydrous THF (10ml) was added Leu-OMe.HCl (0.79g, 4.3mmol) and DIPEA (0.75ml, 4.3mmol). The reaction was allowed to stir at room temperature for 3 days before refluxing for 6 hours. The reaction mixture was then diluted with ether (40ml), the organics were washed with 0.5M HCl (60 ml), dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (85:15) to give alkene (**480**) (0.45g, 25%) and the hydroxymethyl dipeptide (**476**) (0.74g, 31%).

***(2S,3S,2'S)-1-(tert-Butyldiphenylsilyloxy)-2-leucinylmethylester-3-phthaloyl-aminobutane* (476).**

(2*S*,3*S*,2'*S*)-1-*tert*-butyldiphenylsilyloxy-2-leucinylmethylester-3-phthaloylamino-butane (**476**) was isolated as a gum; $[\alpha]_{589}^{18}$ -18.2 (*c* 1.35 in $CHCl_3$).

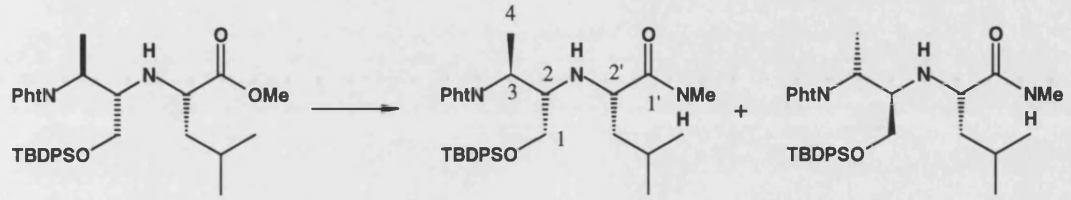
***(3S)-(Z)-1-(tert-Butyldiphenylsilyloxy)-3-phthaloylaminobut-1-ene* (480)**

(3*S*)-(*Z*)-1-(*tert*-butyldiphenylsilyloxy)-3-phthaloylaminobut-1-ene (**480**) was isolated as an oil; $[\alpha]_{589}^{19}$ 8.33 (*c* 1.668 in $CHCl_3$).

***(2S*,3S*,2'S)-1-(tert-Butyldiphenylsilyloxy)-2-leucinylmethylamide-3-phthaloyl-aminobutane* (482) *and* (483).**

To a cooled solution (0°C) of protected esters (**476**) and (**477**) (1.55g, 2.58mmol) in anhydrous methanol (10ml) was bubbled methylamine gas. After 30 mins., the methylamine gas flow was stopped and the reaction mixture was left to stir for 16 hours. After this time the reaction mixture was acidified with 1M HCl (pH~5), neutralised (saturated $NaHCO_3$), concentrated *in vacuo*, extracted with $CHCl_3$ (2 x 80ml), the organics dried ($MgSO_4$) and concentrated *in vacuo* to give a crude material that flash chromatographed, eluting with petrol:EtOAc gradient from (50:50) to EtOAc. Four compounds were isolated of which the desired amides (**482**) and (**483**) was isolated (0.2g, 13%) as a colourless gum; $R_f$ [petrol:ether(50:50)] 0.33; $\nu_{max}/cm^{-1}$ $3407_S$ (NH), $1720_S$ (C=O), $1713_S$ (C=O), $1660_S$ (NC=O), $1470_{\delta\ as}$ (Me), $1384_{\delta\ s}$ (Me) and 1111; $\delta_H$(270 MHz, $CDCl_3$) 0.87 (3H, d, *J* 6.4, CH*Me*$_2$), 0.96 (3H, d, *J* 6.4, CH*Me*$_2$), 1.10 (9H, s, $^t$Bu), 1.33 (3H, d, *J* 7.1, CH*Me*), 1.65-1.83 (3H, m, C*H*$_2$C*H*Me$_2$), 2.62 and 2.64 (3H, s, rotamers, NH*Me*), 3.63 (1H, dd, *J* 10.1 and 8.3, CH*H*OSi), 3.83 (1H, dd, *J* 10.2 and 5.3, C*H*HOSi), 4.17-4.26 (1H, m, C*H*N), 5.00 (1H, dd, *J* 10.2 and 5.3, C*H*CO$_2$Me), 5.05 (1H, brs, NH), 5.17 (1H, dq, *J* 7.3 and 3.0, C*H*Me), 6.20 (1H, s, rotamers, N*H*Me), 7.34-7.50 (5H, m, phenyl), 7.64-7.74 (5H, m, phenyl), 7.90-7.94 (2H, m, phthaloyl) and 8.03-8.08 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 11.72 (CH*Me*), 19.14 (*C*Me$_3$), 21.94 (CH*Me*$_2$), 22.56 (CH*Me*$_2$), 23.18 (*C*HMe$_2$), 24.95 (NHMe), 25.90 (NHMe), 26.78 (C*Me*$_3$), 44.43 [minor isomer (2*R*,3*R*,2'*S*) $CH_2$],

44.76 [major isomer (2*S*,3*S*,2'*S*) $CH_2$], 48.78 (*C*HMe), 62.04 (*C*H$CO_2$), 64.33 [major isomer (2*S*,3*S*,2'*S*) $CH_2O$], 64.44 [minor isomer (2*R*,3*R*,2'*S*) $CH_2O$], 73.49 (CHN), 123.03 (SiPh), 123.74 (SiPh), 125.85 (*m*-C), 127.64 (SiPh), 127.69 (SiPh), 129.14 (*C*C=O), 129.69 (SiPh), 129.76 (SiPh), 132.32 (SiPh), 132.41 (SiPh), 133.88 (SiPh), 133.07 (*o*-C), 135.60 (SiPh), 133.75 (SiPh), 135.28 (SiPh), 135.40 (SiPh), 152.84 (CONMe), 168.20 (CON) and 173.20 (*C*$O_2$Me); m/z (C.I.) 600 ($M^+$+1, 100%); [Found : $(M+1)^+$ 600.3254. $C_{35}H_{46}N_3O_4Si$ requires 600.3257].




**(476)** and **(477)** **(482)** **(483)**

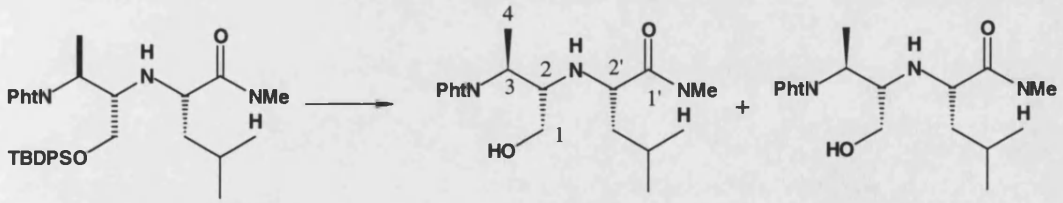
***(2S,3S,2'S)-1-(tert-Butyldiphenylsilyloxy)-2-leucinylmethylamide-3-phthaloyl-aminobutane* (482).**

To the protected ester **(476)** (0.462g, 0.77mmol) was added a diluted solution (5ml) of (33%) methylamine in methylated spirits in anhydrous methanol (2ml:98ml) . The reaction mixture was left to stir for 2 days. After this time the reaction mixture was concentrated *in vacuo* to give a crude material that flash chromatographed, eluting with petrol:EtOAc gradient from (50:50). Four compounds were isolated of which the starting materials **(476)** (0.19g, 41%) and the desired amide **(482)** was isolated (0.1g, 22%) as a colourless gum; $R_f$ [petrol:ether (50:50)] 0.33; $[\alpha]_{589}^{20}$-6.4 (*c* 0.92 in $CHCl_3$).

***(2S,3S,2'S) and (2R,3R,2'S)-1-Hydroxy-2-leucinylmethylamide-3-phthaloyl-aminobutane* (484a) *and* (484b).**

To a cooled solution (0°C) of the protected alcohols **(482)** and **(483)** (0.1g, 0.167mmol) in THF (3ml) was added TBAF (0.33ml, 0.33mmol). The reaction mixture was stirred for 2 hours, diluted with EtOAc (15ml), washed with brine

(20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude alcohols (**484**). This was flash chromatographed, eluting with $CHCl_3$:MeOH (90:10) to give the desired alcohol (**484a**) and (**484b**) (0.04g, 67%); $R_f$ [$CHCl_3$:MeOH (90:10)] 0.23; $\nu_{max}/cm^{-1}$ $3332_S$ (NH), $1725_S$ (C=O), $1660_S$ (NC=O), 1539, $1465_{\delta\ as}$ (Me), 1377 and 1056; $\delta_H$(270 MHz, $CDCl_3$) 0.81 (3H, d, *J* 6.6, CH*Me*$_2$), 0.89 (3H, d, *J* 6.6, CH*Me*$_2$), 1.36 [minor isomer (2*R*,3*R*,2'*S*) 3H, d, *J* 7.0, CH*Me*], 1.44 (3H, d, *J* 7.0, CH*Me*), 1.58-1.70 (1H, m, $CH_2CHMe_2$), 1.72-1.95 (1H, m, $CH_2CHMe_2$), 2.70 and 2.72 (3H, s, rotamers, NH*Me*), 3.51 (1H, dd, *J* 11.7 and 4.0, CH*H*O), 3.61 (1H, s, OH), 3.63 (1H, dd, *J* 11.4 and 4.4, C*H*HO), 4.14 (1H, dd, *J* 5.9 and 4.2, C*H*N), 4.83 (1H, dq, *J* 7.0 and 6.2, C*H*Me), 4.94 (1H, dd, *J* 7.9 and 4.7, C*H*$CO_2$Me), 6.88 (1H, brs, NH), 7.30 (1H, 2 x s, rotamers, N*H*Me), 7.58-7.67 (2H, m, phthaloyl), 7.78-7.84 (1H, m, phthaloyl) and 7.92-7.98 (2H, m, phthaloyl); $\delta_C$ (67.8 MHz, $CDCl_3$) major isomer (2*S*,3*S*,2'*S*) 13.56 (CH*Me*), 22.32 (CH*Me*$_2$), 23.31 (CH*Me*$_2$), 25.10 (*C*H$Me_2$), 26.31 and 26.52 (NHMe), 44.56 ($CH_2$), 48.07 (*C*HMe), 61.71 (*C*H$CO_2$), 63.43 ($CH_2OH$), 73.68 (CHN), 123.74 (*m*-C), 129.16 (*C*C=O), 132.32 (*o*-C), 151.36 (CONMe) and 168.20 (CON); m/z (C.I.) 362 ($M^+$+1, 40%), 331 (5, $M^+$-$CH_2OH$) and 303 (35, $M^+$-C=O$NH_2Me^+$).




**(482) and (483)** **(484a)** **(484b)**

***(2S,3S,2'S)-1-Hydroxy-2-leucinylmethylamide-3-phthaloylaminobutane* (484a)**

To a cooled solution (0°C) of the protected alcohol (**482**) (0.097g, 0.16mmol) in THF (3ml) was added TBAF (0.33ml, 0.33mmol). The reaction mixture was stirred for 2 hours, diluted with EtOAc (15ml), washed with brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude alcohol (**484a**). This was flash chromatographed, eluting with $CHCl_3$:MeOH (90:10) to give the desired

alcohol () (0.045g, 77%); $R_f$ [$CHCl_3$:MeOH(90:10)] 0.23; $[\alpha]_{589}^{20}$ -1.3 (*c* 0.896 in $CHCl_3$).

### 4.2.2 *N,N*-Dibenzylamino protected compounds

#### *N,N*-Dibenzyl-Ala-OBn (359)

**Method A**

To a solution of Ala (10.0g, 112mmol) in EtOH (60ml) and water (60ml) was added BnBr (44ml, 370mmol), $K_2CO_3$ (23.22g, 168mmol) and NaOH (6.72g, 168mmol). The reaction mixture was refluxed for 7 hours or until all the Ala had been consumed. The reaction mixture was diluted with EtOAc (200ml), washed with 1M HCl (100ml), brine (100ml), water (100ml), dried ($MgSO_4$) and concentrated *in vacuo*. Any remaining BnBr or BnOH were distilled off under vacuum to give the desired protected benzyl ester **(360)** in excellent yield (39.15g, 97%).


**(345)** **(359)**

**Method B**

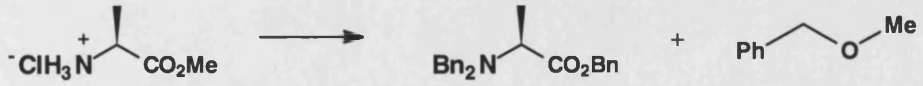
To a solution of alanine (10.0g, 112mmol) in 95% EtOH (200ml) was added BnBr (29.38ml, 247mmol), $K_2CO_3$ (16.30g, 118mmol) and NaOH (4.72g, 118mmol). The reaction mixture was refluxed for 7 hours or until all the alanine had been consumed. The reaction mixture was diluted with EtOAc (200ml), washed with 1M HCl (100ml), brine (100ml), water (100ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. This material was flash chromatographed,

eluting with petrol:EtOAc (90:10) to give the protected benzyl ester **(359)** (10.1g, 25%).

**Attempted preparation of *N,N*-dibenzyl-Ala-OMe (360)**

**Method A**

To a solution of Ala-OMe.HCl **(362)** (15.8g, 113mmol) in MeOH (200ml) was added BnBr (38.7ml, 226mmol), $K_2CO_3$ (23.2g, 170mmol) and NaOH (23.0ml, 170mmol). The reaction mixture was refluxed for 7 hours or until all the alanine had been consumed. The reaction mixture was diluted with EtOAc (200ml), washed with 1M HCl (100ml), brine (100ml), water (100ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product (30.3g). This material was flash chromatographed, eluting with petrol:EtOAc (90:10) to give the protected benzyl ester (-) (30.1g, 73%) and the methylphenylmethyl ether **(360)** (6.9g).




**(362)** **(359)** **(361)**

**Method B**

To a stirred suspension of $K_2CO_3$ (5.43g, 39.3mmol) in acetone (30ml) was added Ala-OMe.HCl **(362)** (1.71g, 12.3mmol), NaI (0.02g, 0.12mmol) and BnBr (4.67ml, 39.3mmol). The reaction mixture was stirred for 2 days, triethylamine (1.7ml, 12.3mmol) was added and the reaction was stirred for 4 hours. The reaction mixture was diluted with 0.1M HCl (30ml), concentrated *in vacuo* until water remained, this was extracted with EtOAc (2 x 50ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product (1.25g). This was flash chromatographed, eluting with petrol:EtOAc (95:5) to give the protected methylester **(360)** (0.383g, 11%) as a colourless oil; $R_f$ [petrol:EtOAc (90:10)] 0.4;

(Found C, 76.62; H, 7.23; N, 4.34. calc. for $C_{18}H_{21}NO_2$ : C, 76.60; H, 7.47; N, 4.94%); $\nu_{max}/cm^{-1}$ $1734_s$ (CO), 1495, $1454_{\delta\ as}$ (Me), 1202 and 1147; $\delta_H$(270 MHz, $CDCl_3$) 1.33 (3H, d, *J* 7.0, CH*Me*), 3.51 (1H, q, *J* 7.0, C*H*Me), 3.62 (2H, d, *J* 14.0, $PhCH_2$), 3.74 (2H, s, OMe), 3.83 (2H, d, *J* 14.0, $PhCH_2$) and 7.20-7.40 (10H, m, phenyl); m/z (C.I.) 284 (100%, $M^+$+1) and 224 ($M^+$-$CO_2Me$, 50).


**(362)** **(360)**

**Method C**

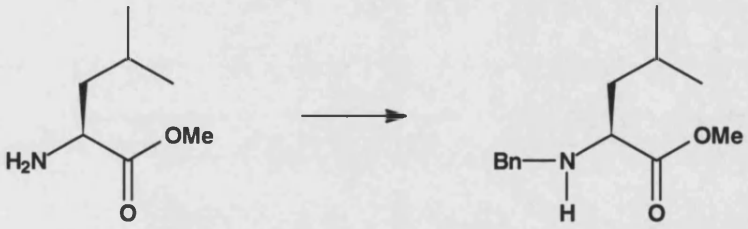
To a stirred suspension of Ala-OMe.HCl **(362)** (7.571g, 54.2mmol) in THF (120ml) was added triethylamine (24.2ml, 173.6mmol) and BnBr (21.3ml, 179mmol). The reaction mixture was stirred for 1 day. The reaction mixture was diluted with 0.1M HCl (100ml), extracted with EtOAc (3 x 150ml), the organics were combined, washed with brine (150ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with petrol:EtOAc (95:5) to give the protected methyl ester **(360)** (4.89g, 32%) as a colourless oil.

**Method D**

To a stirred solution of $Bn_2$-Ala-OBn **(359)** (2.21g, 6.15mmol) in methanol (20ml) was bubbled freshly generated HCl gas (from a Kepp's apparatus). The reaction mixture was stirred for 4 hours whilst HCl gas was passed into the methanol, then a further 4 hours. No benzyl alcohol was observed by t.l.c. The reaction mixture was neutralised with $NaHCO_3$, concentrated *in vacuo*, the residue was diluted with water (100ml), extracted with EtOAc (100ml), dried ($MgSO_4$) and concentrated *in vacuo*, to give recovered starting material (2.1g, 95%).

**mono protected Leu-OMe (367)**

To a stirred suspension of Leu-OMe.HCl (**395**) (1.19g, 6.57mmol) in THF (30ml) and EtOH (3ml) was added $K_2CO_3$ (2.72g, 19.7mmol) and BnBr (0.87ml, 7.3mmol). The reaction mixture was refluxed for 2 days. After cooling, the reaction mixture was diluted with EtOAc (30ml), washed with water (50ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 1.34g of a crude oil. This was flash chromatographed, eluting with petrol:EtOAc (90:10) to give the product (**367**) (0.885g, 57%); $R_f$ [petrol:EtOAc (1:1)] 0.92; (Found C, 71.56; H, 9.08; N, 5.80. calc. for $C_{14}H_{21}NO_2$ : C, 71.46; H, 9.00; N, 5.95%); $\nu_{max}/cm^{-1}$ $3333_s$ (NH), $1737_s$ (C=O), 1466 and 1195; $\delta_H$(270 MHz, $CDCl_3$) 0.84 (3H, d, *J* 6.6, CH*Me*$_2$), 0.91 (3H, d, *J* 6.6, CH*Me*$_2$), 1.44-1.50 (2H, m, $CH_2$), 1.71-1.82 (2H, m, NH and C*H*Me$_2$), 3.31 (1H, t, *J* 7, NCH), 3.61 (1H, d, *J* 13, $CH_2N$), 3.77 (1H, d, *J* 13, $CH_2N$), 3.79 (1H, s, OMe) and 7.24-7.33 (5H, m, phenyl); m/z (C.I.) 236 ($MH^+$).




(**395**) (**367**)

***N,N*-Dibenzyl-alaninol (349)**

To a stirred solution of $LiAlH_4$ (4.85g, 121.2mmol) in THF (80ml) was added $Bn_2$-Ala-OMe (**360**) (29.05g, 80.8mmol) in THF (80ml) at such a rate to maintain reflux. The reaction mixture was refluxed for 6 hours. Cooled to 0°C, quenched with sat. $NH_4Cl$ (3ml), diluted with ether (100ml) and filtered through celite. The residual salts were washed with ether (3 x 150ml), the organics combined, dried ($MgSO_4$) and concentrated *in vacuo* to give crude oil. This was distilled under vacuum (to remove BnOH) to give the product (**349**) (16.7g, 81%); $R_f$ [petrol:EtOAc (1:1)] 0.5; $[\alpha]_{589}^{21}$ 86.5 (*c* 0.614 in $CHCl_3$); $\nu_{max}/cm^{-1}$ $3447_s$ (OH), 1494, $1452_{\delta\ as}$ (Me) and 1043; $\delta_H$(270 MHz, $CDCl_3$) 0.97 (3H, d, *J* 6.6, CH*Me*$_2$),

2.94-3.05 (1H, m, C*H*Me), 3.20 (1H, br s, OH), 3.30-3.50 (4H, m, $CH_2N$, $CH_2OH$), 3.82 (2H, d, *J* 13.4, $CH_2N$) and 7.21-7.35 (10H, m, phenyl).


**(359)** **(349)**

***N,N*-Dibenzyl-Ala-H (358)**

**Method A**

To a solution of alcohol **(349)** (4.82g, 19mmol) in DCM (150ml) under $N_2$ was added celite (8g) and PCC (8.13g, 38mmol). After refluxing for 1 hour the reaction mixture was allowed to cool before being diluted with ether (400ml). This was then filtered through Florisil (300g) and the residue washed with ether (4 x 400ml). The combined organics were concentrated *in vacuo* to give 0.6g of crude material. T.l.c. showed three compounds when visualised by short wavelength light, these were benzaldehyde, starting material and the desired aldehyde **(358)** (0.433g, 9%) as a colourless oil; $R_f$ [petrol:EtOAc (85:15)] 0.5; $\nu_{max}/cm^{-1}$ $1727_s$ (CO), 1495 and $1453_{\delta\ as}$ (Me); $\delta_H$(270 MHz, $CDCl_3$) 1.00 (3H, d, *J* 7.0, CH*Me*), 3.50-4.00 [5H, m, $(PhCH_2)_2N$, C*H*Me], 7.21-7.35 (10H, m, phenyl) and 9.7 (1H, s, CHO); m/z (C.I) 254 ($M^+$+1, 40%) and 224 (20, $M^+$-$OCH_3$).


**(349)** **(358)**

**Method B**

To a solution of alcohol **(349)** (1.41g, 5.52mmol) in DCM (20ml) under $N_2$ was added NMO (0.97g, 8.28mmol), powdered 4Å molecular sieves and TPAP (0.098g, 0.28mmol). After stirring at room temperature for 2 hours the reaction

mixture was flash chromatographed, eluting with petrol:ether (10:90) to afford the aldehyde **(358)** (1.0g, 72%) as a colourless oil.

**Method C**

A solution of oxalyl chloride (3.18ml, 34.6mmol) in DCM (60ml) was cooled to -78°C. To this was added DMSO (4.92ml, 63.6mmol) in DCM (15ml) at such a rate as to maintain the temperature between -60 to -50°C. The reaction was then stirred for 2 minutes before the addition of the alcohol **(349)** (8.12g, 31.8mmol) in DCM (25ml). The reaction mixture was then stirred for 20 minutes at -78°C before the addition of triethylamine (22.3ml, 159mmol). After 5 minutes the reaction mixture was allowed to attain room temperature and then quenched with water (20ml), washed with 1% HCl (100ml), water (100ml), 5% $Na_2CO_3$ (100ml), water (100ml) and concentrated *in vacuo* to afford aldehyde **(358)** (7.18g, 89%) as a colourless oil.

**DiBAL-H reduction of *N,N*-dibenzyl-Ala-OBn (359)**

To cooled (-78°C) solution of *N,N*-dibenzyl-Ala-OBn **(359)** (4.02g, 11.2mmol) in toluene (30ml) was added 1.0M DiBAL-H (13.5ml, 13.5mmol) over 30 minutes. The reaction mixture was stirred for 2 hours. The reaction was quenched with MeOH (3ml), diluted with Rochelle salts (33ml of sat. in 200ml) and stirred at room temperature for 1 hour. The reaction mixture was diluted with ether (100ml) and filtered through celite. The residual salts were washed with ether (3 x 150ml), the organics combined, dried ($MgSO_4$) and concentrated *in vacuo* to give a crude oil. This was flash chromatographed, eluting with petrol:EtOAc (95:5) to give the product **(358)** (0.43g, 15%).


**(359)** **(358)**

**DiBAL-H reduction of *N,N*-dibenzyl-Ala-OMe (360)**

To cooled (-78°C) solution of *N,N*-dibenzyl-Ala-OMe **(360)** (5.21g, 18.4mmol) in toluene (30ml) was added 1.0M DiBAL-H (38.6ml, 38.6mmol) over 30 minutes. The reaction mixture was stirred for 1 hour. The reaction was quenched with MeOH (1.5ml) and poured onto Rochelle salts (15ml of sat. in 100ml of water) and stirred at 0°C for 1 hour. The reaction mixture was diluted with ether (50ml) and filtered through celite. The residual salts were washed with ether (5 x 50ml), the organics combined, dried ($MgSO_4$) and concentrated *in vacuo* to give only alcohol **(349)** (1.91g, 40%).

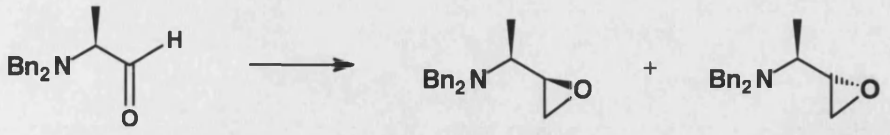

**(360)** **(349)**

**(2*R*) and (2*S*)-[1*S*-*N,N*-Dibenzylaminoethyl]-oxirane (356) and (357)**

**Method A**

To a solution of aldehyde **(358)** (0.33g, 1.3mmol) and TBAI (0.005g, 0.015mmol) in DCM (10ml) was added 50% aqueous NaOH (0.3ml) and trimethylsulfonium iodide (0.27g, 1.3mmol). The reaction mixture was stirred at 50°C for 1 hour. T.l.c. showed that all the starting material had been consumed, the reaction was poured onto ice, the organic phase was separated, washed with water (10ml), dried ($MgSO_4$) and concentrated *in vacuo.* The crude material was flash chromatographed, eluting with EtOAc:petrol (15:85) to give an inseparable mixture of the epoxides **(356)** and **(357)** (0.04g, 12%), $R_f$ [petrol:EtOAc (85:15)] 0.38; as a colourless oil; (Found C, 80.61; H, 8.00; N, 4.78. calc. for $C_{18}H_{21}NO$ : C, 80.86; H, 7.92; N, 5.24%); $\nu_{max}/cm^{-1}$ 1493, $1453_{\delta\ as}$ (Me), $1369_{\delta\ s}$ (Me), $1260_s$ (C-O-C) and $850_s$ (C-O-C); $\delta_H$(270 MHz, $CDCl_3$) for major isomer **(356)**, 1.03 (3H, d, *J* 6.6, CH*Me*), 2.41 (1H, dd, *J* 4.8 and 2.9, C*H*HO), and minor isomer **(357)**, 2.48-2.55 (1H, m, CHO), 2.66 (1H, dd, *J* 5.1 and 4.0, CH*H*O), 2.79 (1H, qd,

*J* 6.6 and 4.4, C*H*Me), 3.08 (1H, ddd, *J* 7.3, 4.4 and 2.9, CHO), 3.60 (2H, *J* 14.3, $CH_2N$), 3.80 (2H, d, *J* 14.3, $CH_2N$) and 7.17-7.40 (10H, m, phenyl); m/z (C.I.) 268 ($M^+$+1, 50%).




**(358)** **(356)** **(357)**

**Method B**

To NaH (0.39g, 13mmol) was added DMSO (20ml) the solution was heated to 75-80°C for 45 minutes. The reaction mixture was allowed to attain room temperature, THF (40ml) was added and the reaction mixture was cooled (-10°C). To the rapidly stirred mixture, a solution of trimethylsulfonium iodide (2.6g, 12.5mmol) in DMSO (20ml) was added. After 5 minutes the aldehyde **(358)** (3.0g, 12mmol) in THF (10ml) was added and stirred at -10°C for 30 mins and a further 1 hour at room temperature. The reaction mixture was quenched with water (250ml), extracted with ether (3 x 250ml), the organics were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (90:10) to give **(356)** and **(357)** (87:13) (2.4g, 76%).

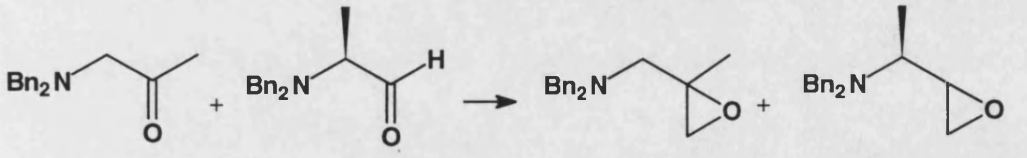
***Preparation of (2R) and (2S)-methyl-[(1S)-N,N-dibenzylamino-1-methyl]-oxirane* (363).**

To NaH (0.147g, 4.9mmol) was added DMSO (10ml) the solution was heated to 75-80°C for 45 minutes. The reaction mixture was allowed to attain room temperature, THF (20ml) was added and the reaction mixture was cooled (-10°C). To the rapidly stirred mixture, a solution of trimethylsulfonium iodide (0.96g, 4.7mmol) in DMSO (10ml). After 5 minutes aldehydes **(358)** and **(363)** (1.13g, 4.5mmol) in THF (5ml) were added and stirred at -10°C for 30 mins and a further 1 hour at room temperature. The reaction mixture was quenched with water (100ml),

extracted with ether (3 x 150ml), the organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.99g of crude material. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (95:5) to give an inseparable mixture of the epoxides **(356)** and **(357)** and the epoxides **(363)**; (**356**,**357**:**363**, 31:69) (0.73g, 61%); $R_f$ [petrol:EtOAc (85:15)] 0.38:

***(2R) and (2S)-methyl-[(1S)-N,N-dibenzylamino-1-methyl]-oxirane* (363).**

(2*R*) and (2*S*)-methyl-[(1*S*)-N,N-dibenzylamino-1-methyl]-oxirane **(363)** was isolated as a colourless oil; (Found C, 80.80; H, 8.29; N, 5.14. calc. for $C_{18}H_{21}NO$ : C, 80.86; H, 7.92; N, 5.24%); $\nu_{max}/cm^{-1}$ 1682, 1493, $1453_{\delta\ as}$ (Me), $1369_{\delta\ s}$ (Me), $1260_s$ (C-O-C) and $850_s$ (C-O-C); $\delta_H$(270 MHz, $CDCl_3$) 1.39 (3H, s, CH*Me*), 2.36 (1H, d, *J* 13.4, C*H*HN), 2.52-2.57 (2H, m, $CH_2O$), 2.62 (1H, d, *J* 13.2, C*H*HN), 3.46 (2H, d, *J* 13.5, PhC$H_2$N), 3.73 (2H, d, *J* 13.5, PhC$H_2$N) and 7.20-7.40 (10H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 19.0 (Me), 52.0 ($CH_2O$), 56.5 (CHMe), 59.0 (Ph$CH_2$N), 59.5 ($CH_2$N), 127 (*p*-phenyl), 128.5 (phenyl), 129.0 (phenyl) and 139.0 (phenyl-$CH_2$); m/z (E.I 70eV) 267 ($M^+$, 5%) and 210 (50, $M^+$-$CH_3C_2H_2O$); (C.I.) 268 ($M^+$+1, 70%) and 210 (50, $M^+$-$CH_3C_2H_2O$).



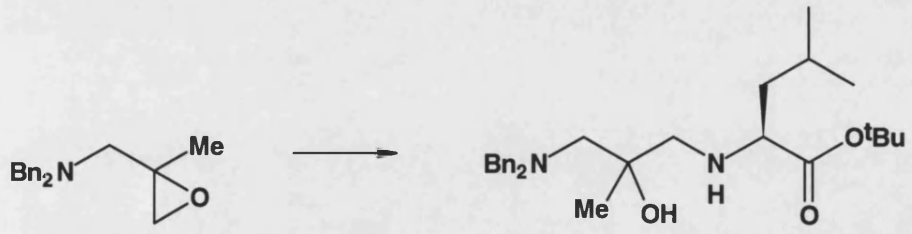

**(366)** **(358)** **(363)** **(356)** and **(357)**

***Attempted preparation of (2S,3S,2'S)- and (2R,3S,2'S)-3-N,N-dibenzylamino-1-hydroxy-2-(O-methyl leucinyl)butane* (401).**

To a cooled solution (-78°C) of epoxides **(356)** and **(357)** (0.441g, 1.65mmol) in anhydrous DCM (7ml) was added $BF_3.OEt_2$ (0.193ml, 1.57mmol) and Leu-OMe generated by reacting Leu-OMe.HCl (0.60g, 3.3mmol) with triethylamine (0.46ml, 3.3mmol) in THF, and then filtered before addition. By t.l.c. there were a vast number of spots, so we abandoned the reaction.

***(2S,3S,2'S)- and (2R,3S,2'S)-3-N,N-dibenzylamino-2-hydroxy-2-methyl-1-(O-tert-butyl leucinyl) propane* (402).**

To a solution of *N,N*-dibenzylamino epoxide **(363)** (0.32g, 1.2mmol) in MeOH (10ml), was added Leu-O$^t$Bu.HCl (0.35g, 1.56mmol) and triethylamine (0.217ml, 1.56mmol). The reaction mixture was refluxed for 1 day, cooled, concentrated *in vacuo* and diluted with EtOAc (20ml). The organics were washed with water (20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 0.39g of crude material. This was flash chromatographed, eluting with petrol:EtOAc (90:10), to yield starting materials **(363)** (0.16g, 50%) and the amino alcohol **(402)** (0.11g, 20%) as a colourless oil $R_f$ [petrol:EtOAc (80:20) ] 0.35; $\nu_{max}/cm^{-1}$ $3442_s$ (OH), $1726_s$ (C=O), $1455_{\delta\ as}$ (Me), $1368_{\delta\ s}$ (Me), $1250_s$ (C-N) and 1151; $\delta_H$(270 MHz, $CDCl_3$) 0.80-0.90 (6H, m, CH*Me*$_2$), 0.99 (3H, s, Me), 1.30-1.70 (3H, m, C*H*$_2$C*H*Me$_2$), 1.38 (9H, s, $^t$Bu), 1.97-2.09 (2H, m, $CH_2$-Leu), 2.35-2.47 (2H, m, $CH_2NBn_2$), 3.60 [4H, d, *J* 12.1, $(PhCH_2)_2N$], 3.70-3.80 (1H, m, NCHCO) and 7.18-7.30 (10H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 22.17 (CH*Me*$_2$), 22.64 (*C*Me$_3$), 22.77 (CH*Me*$_2$), 24.81 (*C*HMe$_2$), 25.25 (Me), 42.75 (*C*H$_2$CHMe$_2$), 56.24 ($Bn_2NCH_2$), 60.26 ($CH_2$-Leu) 61.48 (N*C*HCO), 80.75 [*C*(Me)OH], 126.90 (*p*-phenyl), 128.28 (phenyl), 128.45 (phenyl), 128.51 (phenyl), 128.99 (phenyl), 139.57 (phenyl-$CH_2N$) and 175.07 ($CO_2$$^t$Bu); m/z (C.I.) 455 ($M^+$+1, 100%); [Found : ($M^+$+1) 455.3277. $C_{28}H_{43}N_2O_3$ requires 455.3274].

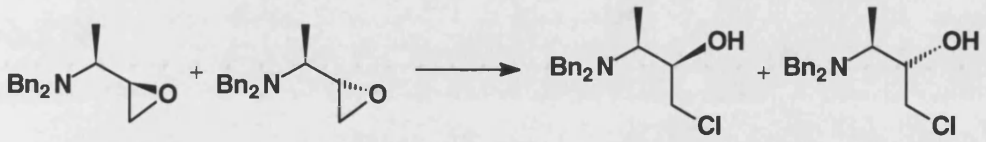



**(363)** **(402)**

***(2S,3S) and (2R,3S)-3-N,N-Dibenzylamino-1-chloro-2-hydroxybutane* (407).**

To a stirred solution of epoxides (**356**:**357**, 87:13) (2.3g, 8.6mmol) in THF (80ml) was added lithium chloride (1.75g, 42.0mmol) and acetic acid (1.5ml, 25.8mmol).

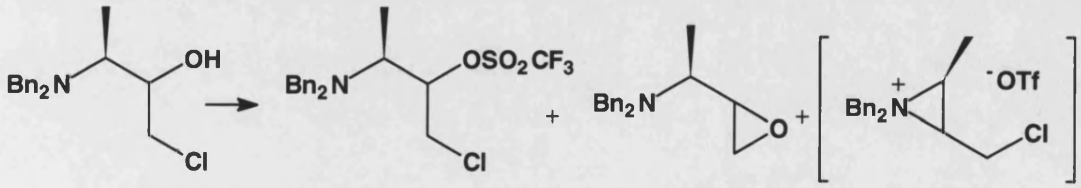
After stirring the mixture at room temperature for 1 day the reaction mixture was diluted with EtOAc (80ml), washed with water (3 x 100ml), dried ($Na_2SO_4$) and concentrated *in vacuo* to afford a quantitative yield of chloroalcohols (**407a:407b**, 87:13) (2.6g) as a colourless oil; $R_f$ [petrol:EtOAc (70:30)] 0.5; $\delta_H$(270 MHz, $CDCl_3$) 1.25 (3H, d, *J* 6.8, Me), 3.44 (2H, d, *J* 13.5, PhC$H_2$N), 3.50-3.70 (2H, m, $CH_2Cl$), 4.01 (1H, br s, OH), 4.27 (1H, dq, *J* 6.9 and 6.8, C*H*Me), 4.50-4.58 (1H, m, CHO), 3.73 (2H, d, *J* 13.5, PhC$H_2$N) and 7.22-7.40 (10H, m, phenyl).




**(356)** **(357)** **(407a)** **(407b)**

***Attempted preparation of (2S,3S)- and (2R,3S)-3-N,N-dibenzylamino-1-chloro-2-(trifluoromethanesulfonyloxy)butane* (471)**

To a cooled solution (-20°C) of the chloroalcohol **(407)** (2.6g, 8.6mmol) in DCM (100ml) was added pyridine (0.87ml, 10.8mmol). The reaction was stirred for 5 minutes before the addition of triflic anhydride (1.97ml, 11.7mmol). It was then allowed to stir at -20°C for 5 minutes before being warmed to room temperature and stirred for a further 1 hour. The reaction mixture was diluted with DCM (50ml), washed with 1M HCl (150ml), brine (150ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 2.7g of a crude red oil. T.l.c. showed a huge number of spots and the crude mixture was not purified.




**(407)** **(471)** **(356)** and **(357)** **(473)**

***(2R,3S)- and (2S,3S)-3-N,N-Dibenzylamino-2-hydroxy-1-triphenylmethylthio-butane* (408).**

To a stirred solution of the epoxides (0.112g, 0.42mmol) in anhydrous methanol (5ml) was added triphenylmethylthiol (0.348g, 1.26mmol) and triethylamine (0.23ml, 1.68mmol). The reaction was then stirred for 3 hours at room temperature. The reaction mixture was filtered to remove the excess triphenylmethylthiol and concentrated *in vacuo*. The resulting solid was dissolved in EtOAc (20ml), washed with water (20ml), brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with cyclohexane:EtOAc (95:5) to give epoxides **(356)** and **(357)** (0.03g, 27%) and an inseparable mix of the desired products (**408a**:**408b**, 38:62) (0.02g, 9%); as a colourless oil; $R_f$[petrol:EtOAc (90:10)] 0.16; $\nu_{max}/cm^{-1}$ $3444_s$ (OH) and $1494_{\delta\ as}$ (Me); $\delta_H$(270 MHz, $CDCl_3$) 0.99 (3H, d, *J* 6.8, Me), 1.60 (1H, br s, OH), 2.24 (1H, dd, *J* 13.0 and 10.0, C*H*OH), 2.44 (1H, dd, *J* 7.3 and 6.7, C*H*Me), 2.90 (1H, dd, *J* 13.0 and 2.5, C*H*H*S*), 3.10-3.30 (1H, m, CH*H*S), 3.26 (2H, d, *J* 13.8, $NCH_2Ph$), 3.51 (2H, d, *J* 13.8, $NCH_2Ph$) and 7.10-7.50 (25H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 8.51 (Me), 38.63 ($CH_2S$), 54.22 ($CH_2Ph$), 57.53 (N*C*HMe), 71.47 (CHOH), 126.69-130.30 (phenyl, 11C), 139.69 (phenyl-$CH_2$PhN) and 144.84 (*C*$Ph_3$).

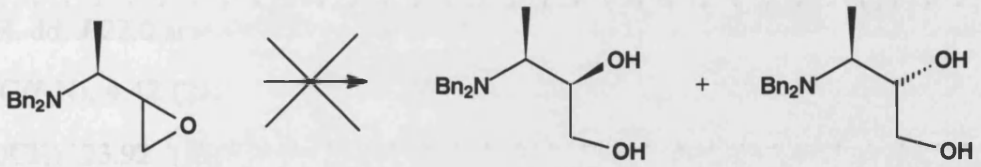

**(356)** and **(357)** **(408)**

***Attempted preparation of the (2S,3S)- and (2R,3S)-3-N,N-dibenzylamino-2-dihydroxybutane* (411).**

**Method A**

To a cooled solution (0°C) of the epoxides (**356**:**357**, 87:13) (0.20g, 1.0mmol) in DCM (2ml) was added $BF_3.OEt_2$ (0.11ml, 1.1mmol). After 1 minute, water

(0.1ml) was added. The reaction was stirred at 0°C for 10 minutes before being warmed to room temperature and stirred for a further 2 hours. After this time the starting material had been consumed and the reaction was quenched with 2M HCl (5ml) and extracted with DCM (2 x 10ml). The organics were dried ($Na_2SO_4$) and concentrated *in vacuo* to afford 0.207g crude material. This was flash chromatographed on silica, eluting with petrol:EtOAc (50:50) to give an unknown material which was highly deliquescent, we did not determine the structure of this compound.




**(356)** and **(357)** **(411a)** **(411b)**

**Method B**

To a stirred solution of epoxides (**356**:**357**, 87:13) (0.055g, 2.06mmol) in THF:water (20ml:5ml) was added Dowex-50X8-100 ion-exchange resin (strongly acidic). The reaction mixture was stirred at 50°C for 11 hours, cooled and filtered. The resin was washed with $CHCl_3$ (50ml), concentrated *in vacuo* and crystallised from hexane:EtOAc to afford 0.03g of unknown material which we could not identify.

***(2R,3S)- and (2S,3S)-3-N,N-Dibenzylamino-2-hydroxy-2-methylpropoxymethyl-phenyl* (413).**

**Method A**

A suspension of 80% NaH in mineral oil (0.032g, 1.06mmol) was washed with pentane. To this was added DMF (15ml) and the reaction mixture was cooled (0° C). To this solution was added benzyl alcohol (0.11ml, 1.06mmol) dropwise and

epoxides **(363)** (0.95g, 0.355mmol) in DMF (5ml). The reaction mixture was heated to 100°C for 10 hour, cooled, and partitioned between water (20ml) and ether (20ml). The aqueous layer was extracted with ether (2 x 15ml), the organics combined, washed with water (15ml), dried ($MgSO_4$) and concentrated *in vacuo.* The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (80:20) to give a racemic mixture of the ethers **(413)** (0.05g, 38%); $R_f$ [petrol:EtOAc (80:20)] 0.51; as a colourless oil; $\nu_{max}/cm^{-1}$ $3464_s$ (OH), 1494, $1453_{\delta\ as}$ (Me), $1369_{\delta\ s}$ (Me) and 1094; $\delta_H$(270 MHz, $CDCl_3$) 1.09 (3H, s, CH*Me*), 2.53 (1H, d, *J* 13.9, C*H*HN), 2.73 (1H, d, *J* 13.9, CH*H*N), 2.84 (1H,br s, OH), 3.24 (2H, dd, *J* 22.0 and 9.0, $CH_2OBn$), 3.60 (2H, d, *J* 13.6, PhC$H_2$N), 3.70 (2H, *J* 13.6, PhC$H_2$N), 4.42 (2H, s, $OCH_2Ph$) and 7.22-7.30 (15H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 23.92 (Me), 59.91 ($CH_2N$), 60.04 (Ph*C*$H_2$N), 71.86 ($CH_2O$), 73.29 ($OCH_2Ph$), 76.00 [*C*(Me)OH], 127.03 (*p*-phenyl), 127.52 (phenyl), 127.58 (phenyl), 128.26 (phenyl), 129.03 (phenyl), 138 (phenyl-$CH_2O$) and 139.39 (phenyl-$CH_2N$); m/z (C.I.) 376 ($M^+$+1, 70%); [Found : ($M^+$+1) 376.2276. $C_{25}H_{30}NO_2$ requires 376.2276].


**(363)** **(413)**

**Method B**

To a stirred suspension of $Al_2O_3$ (0.5g) in toluene (20ml) was added benzyl alcohol (1.05ml, 10.1mmol) and the epoxide () (0.54g, 2.02mmol). The reaction mixture was stirred for 1 day before refluxing for 4 hours. The reaction mixture was concentrated *in vacuo* and flash chromatographed, eluting with petrol:EtOAc (80:20) to give the starting material and the desired ethers **(413)** (0.014g, 2%).

***1-(diethylphosphonate)-2-phenylethan-2-ol* (430).**

To a solution of diethyl methylphosphonate (0.37ml, 2.46mmol) in THF (20ml) at -78°C was added 1.6M *n*-BuLi (1.44ml, 2.3mmol) dropwise. The reaction mixture was stirred under an inert atmosphere of nitrogen for 30 minutes before the addition of benzaldehyde (0.52g, 4.9mmol). The reaction mixture was stirred at -78°C for 30 minutes, no reaction occurred, so the reaction mixture was allowed to stir overnight. The reaction mixture was then quenched with water (20ml), diluted with EtOAc (20ml), extracted, washed with brine (30ml), the organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 0.9g of crude yellow oil. This was flash chromatographed, eluting with EtOAc to give the β-hydroxyphosphonate **(430)** (0.68g, 54%) as a colourless oil; $R_f$ [EtOAc] 0.36; (Found C, 55.50; H, 7.42. calc. for $C_{12}H_{19}NO_4P$ : C, 55.80; H, 7.40%); $\nu_{max}/cm^{-1}$ $3288_s$ (OH), $1221_s$ (C-O), $1028_s$ (P-O) and 960; $\delta_H$(270 MHz, $CDCl_3$) 1.29 (3H, t, *J* 7.0, $CH_2CH_3$), 1.62 (3H, t, *J* 7.0, $CH_2CH_3$), 2.26-2.4 (2H, m, $CH_2P$=0), 3.95 (1H, d, *J* 2.6, OH), 4.09 (4H, qm, *J* 7, $CH_2CH_3$), 5.12 (1H, tm, *J* 11.2, CHOH) and 7.24-7.42 (5H, m, phenyl); $\delta_P$(109.25MHz, $CDCl_3$) 29.84; $\delta_C$ (67.8 MHz, $CDCl_3$) 16.4 ($CH_2CH_3$), 34.9 and 36.9 ($CH_2P$), 61.9 and 62.0 (d, *J* 7.5, $CH_2CH_3$), 68.7 and 68.8 (CHOH), 125.5 (*p*-phenyl), 127.7 (*o*-phenyl), 128.5 (*m*-phenyl) and 143.4 and 143.6 (phenyl); m/z (70eV) 258 ($M^+$, 23%), (C.I.) 259 ($M^+$+1, 80) and 241 (100, $M^+$-OH).


**(430)**

***Attempted preparation of the 1-(diethylphosphonate)-2-phenyl-2-(trifluoromethane sulfonyloxy)ethane* (429).**

To a cooled solution (-23°C) of β-hydroxyphosphonate **(429)** (0.276ml, 1.07mmol) in DCM (3ml) was added pyridine (0.063ml, 0.78mmol) dropwise. After 5

minutes triflic anhydride was added, the solution immediately turned pink and then yellow. The reaction mixture was left for 5 minutes before allowing to warm to room temperature. After 10 minutes the reaction mixture was diluted with DCM (15ml), washed with 0.5M HCl (20ml), the acid phase was extracted with DCM (10ml), the organics were combined, dried ($MgSO_4$) and filtered through a plug of flash silica and concentrated *in vacuo* to give mostly the (*E*)-alkene **(431)** (0.23g, 90%);

***(E)-1-diethylphosphonate-2-phenylethene* (431).**

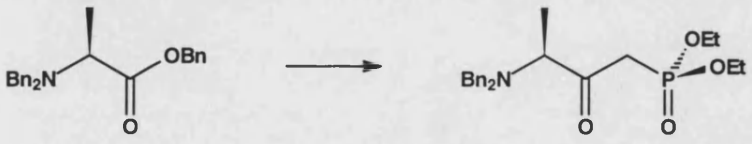
(*E*)-1-diethylphosphonate-2-phenylethene **(431)** was isolated as a brown oil; $R_f$ [EtOAc] 0.6; (Found C, 56.7; H, 7.13. calc. for $C_{12}H_{17}NO_3P$ : C, 56.67; H, 6.74%); $\nu_{max}/cm^{-1}$ 2919, $1649_s$ (C=C), 1496, $1455\delta_{as}$ (Me), $1249_s$ (P=O) and $1027_s$ (P-O); $\delta_H$(270 MHz, $CDCl_3$) 1.36 (6H, t, *J* 6.7, $CH_2CH_3$), 4.14 (4H, qdd, *J* 7, 1.1 and 1.0, $CH_2CH_3$), 6.26 (1H, t, *J* 17.7, PhHC=C*H*P), 7.4-7.51 (5H, m, phenyl) and 7.51 (1H, t, *J* 20, Ph*H*C=CHP); $\delta_C$ (67.8 MHz, $CDCl_3$) 16.3 and 16.4 ($CH_2CH_3$), 61.85 and 61.93 (d, *J* 7, $CH_2CH_3$), 112.4 and 115.3 (CHP), 127.7 (*p*-phenyl), 128.8 (*o*-phenyl), 130.3 (*m*-phenyl), 134.67 and 135.03 (phenyl) and 148.84 and 148.75 (*C*HPh); m/z (70eV) 240 ($M^+$, 20%), (C.I.) 241 ($M^+$+1, 100) and 241 (10, $M^+$-OEt).


**(429)** **(431)**

***Preparation of 3(S)-N,N-dibenzylamino-1-(diethylphosphonate)butan-2-one* (432).**

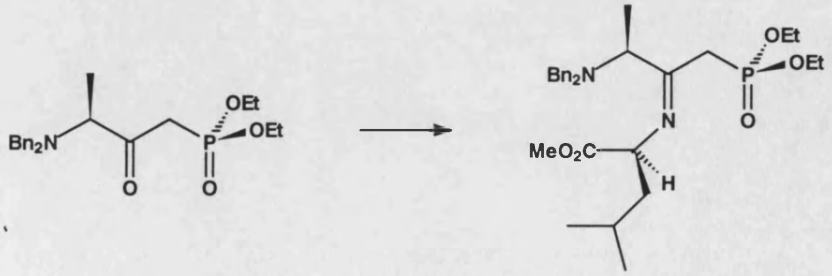
To a solution of diethyl methylphosphonate (5.66ml, 38.76mmol) in THF (150ml) at -78°C was added 1.6M *n*-BuLi (24.23ml, 38.76mmol) dropwise. The reaction mixture was stirred under an inert atmosphere of $N_2$ for 30 minutes before the

addition of the protected amino benzyl ester **(359)** (2.32g, 6.46mmol) in THF (15ml). The reaction mixture was stirred at -78°C for 2 hours, and then at -30°C for 1 hour before the addition of acetic acid (1.8ml). The reaction mixture was then poured onto sat. solution of $NaHCO_3$ (100ml), extracted with EtOAc (2 x 150ml), the organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 4.38g of crude yellow oil. This was flash chromatographed, eluting with petrol:EtOAc (1:1) to give the β-ketophosphonate **(432)** (2.51g, 96%) as a colourless oil; $R_f$ [petrol:EtOAc] 0.5; $[\alpha]_{589}^{20}$ -67.5 (*c* 0.237 in $CH_3Cl$); $\nu_{max}/cm^{-1}$ 3288, 2979, $1718_s$ (C=O), 1494, $1454_{\delta\ as}$ (Me), $1370_{\delta\ s}$ (Me), $1256_s$ (P=O), $1058_s$ (P-O) and 1024; $\delta_H$(270 MHz, $CDCl_3$) 1.14 (3H, t, *J* 7.0, $CH_2CH_3$), 1.18 (3H, d, *J* 6.6, CH*Me*), 1.25 (3H, t, *J* 7.0, $CH_2CH_3$), 2.97 (1H, dd, *J* 22.9 and 14.5, $CH_2P$=0), 3.43 (2H, d, *J* 13.5, $CH_2N$), 3.55 (1H, q, *J* 6.6, C*H*Me), 3.68 (2H, d, *J* 13.5, $CH_2N$), 3.72 (1H, dd, *J* 20.7 and 14.1, $CH_2P$), 3.8-4.0 (2H, m, $POCH_2$), 4.02 (2H, dq, *J* 8.2 and 7.1, $POCH_2$) and 7.22-7.36 (10H, m, phenyl); $\delta_P$(109.25MHz, $CDCl_3$) 20.75; $\delta_C$ (67.8 MHz, $CDCl_3$) 6.5 (CH*Me*), 16.1 and 16.2 ($CH_2CH_3$), 36.7 and 38.6 ($CH_2P$), 57.6 ($CH_2N$), 62.1 and 62.2 ($CH_2CH_3$), 62.3 and 62.7 (*C*HMe), 127.3 (*p*-phenyl), 128.5 (*o*-phenyl), 128.9 (*m*-phenyl), 138.87 (phenyl) and 203.2 and 203.3 (C=O); m/z (C.I.) 404 ($M^+$+1, 100); [Found : ($M^+$+1) 404.19907. $C_{22}H_{31}NO_4P$ requires 404.19907].




**(359)** **(432)**

***Attempted preparation of the imine* (433).**




**(432)** **(433)**

**Method A**

To the β-ketophosphonate **(432)** (1.1g, 2.73mmol) in toluene (50ml) was added Leu-OMe.HCl (0.59g, 3.27mmol) and triethylamine (0.45ml, 3.27mmol). The reaction was refluxed under an inert atmosphere of nitrogen for 6 hours, a new spot was observed by t.l.c. The reaction mixture was concentrated *in vacuo* and then flash chromatographed, eluting with petrol:EtOAc (70:30) to give recovered starting material and a Leucine trimer.

**Method B**

To the β-ketophosphonate **(432)** (0.89g, 2.2mmol) in toluene (50ml) was added Leu-OMe.HCl (0.48g, 2.6mmol) and *p*-toluenesulfonic acid mono hydrate (0.038g, 0.2mmol). The reaction was refluxed under an inert atmosphere of nitrogen for 6 hours, a plethora of spots were observed by t.l.c. and the reaction was abandoned.

***Attempted preparation of (2R,3S,2'S) and (2S,3S,2'S)-3-N,N-dibenzylamino-1-diethylphosphonate-2-(O-methyl leucinyl)butane* (434) *from the ketone* (432).**


**(432)** **(434)**

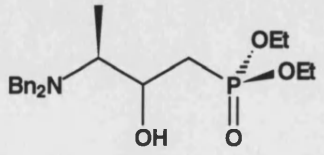
**Method A**

To a stirred solution of the β-ketophosphonate **(432)** (1.85g, 4.59mmol) in methanol (50ml) was added Leu-OMe.HCl (1.67g, 9.17mmol), $NaCNBH_3$ (0.304g, 4.6mmol) and NaOAc (0.384g, 4.59mmol). The reaction was stirred at room temperature under an inert atmosphere of nitrogen for 7 days. After this time no

reaction had occurred, the reaction was left for 2 weeks, after which time all the starting material had been consumed. The reaction mixture was acidified to pH ~2 using 2M HCl, basified to pH~9 with a saturated solution of $NaHCO_3$, extracted with EtOAc (2 x 50ml), the organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.44g of crude colourless oil. This was flash chromatographed, eluting with petrol:EtOAc (40:60) to give the β-hydroxyphosphonate **(436)** (1.4g, 80%) as a colourless oil:

***(2R,3S)- and (2S,3S)-3-N,N-Dibenzylamino-1-(diethylphosphonate)-2-hydroxy-butane* (436).**

(2*R*,3*S*)- and (2*S*,3*S*)-3-*N*,*N*-Dibenzylamino-1-(diethylphosphonate)-2-hydroxybutane **(436)** were isolated as a colourless oil; $R_f$ [DCM:MeOH (95:5)] 0.3; (Found C, 65.0; H, 7.98; N, 3.6. calc. for $C_{22}H_{32}NO_4P$ : C, 65.15; H, 7.95; N, 3.45%); $\nu_{max}/cm^{-1}$ $3370_s$ (OH), 2980, 1603, 1494, $1454_{\delta\ as}$ (Me), $1388_{\delta\ s}$ (Me), $1250_s$ (P=O), 1227 and 1160; $\delta_H$(270 MHz, $CDCl_3$) 1.06 (3H, d, *J* 6.8, CH*Me*), 1.28 (3H, t, *J* 7.0, $CH_2CH_3$), 1.29 (3H, t, *J* 7.0, $CH_2CH_3$), 1.77 (1H, dd, *J* 15.2 and 9.3, $CH_2P$), 1.86 (1H, dd, *J* 15.2 and 2.5, $CH_2P$), 2.56-2.68 (1H, m, C*H*OH), 3.31 (2H, d, *J* 13.5, $CH_2N$), 3.85 (2H, d, *J* 13.5, $CH_2N$), 3.86 (1H, m, C*H*Me), 4.05 and 4.12 (2H, dq, *J* 7.1 and 7.1, $POCH_2$), 4.4 (1H, br s, OH) and 7.22-7.8 (10H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 8.1 (CH*Me*), 16.3 and 16.4 ($CH_2CH_3$), 29.97 and 32.0 ($CH_2P$), 53.5 ($CH_2N$), 58.7 and 59.0 (*C*HMe), 61.4 and 61.8 ($CH_2CH_3$), 67.01 and 67.09 (CHOH), 127.2 (*p*-phenyl), 128.4 (*o*-phenyl), 129.0 (*m*-phenyl) and 138.8 (phenyl); m/z (C.I.) 406 ($M^+$+1, 100%)




**(436)**

**Method B**

To a stirred solution of the β-ketophosphonate **(432)** (2.63g, 6.51mmol) in DCE (25ml) was added Leu-OMe.HCl (1.3g, 7.16mmol), $Na(OAc)_3BH$ (2.07g, 9.8mmol) and triethylamine (1.0ml, 7.16mmol). The reaction was stirred at room temperature under an inert atmosphere of $N_2$ for 10 minutes, no reaction occurred so acetic acid (0.373ml) was added and the reaction mixture was heated to 50°C for 5 hours. The starting material was slowly converted to new compound with very similar $R_f$ to that of the β-hydroxyphosphonate **(432)**, (Md ammonium dip also showed the presence of phosphorus). The reaction was left for two weeks at room temperature. The reaction mixture was basified to pH~9 with a saturated solution of $NaHCO_3$, acidified with 1M HCl, neutralised with 1M NaOH, extracted with $CH_3Cl$ (2 x 50ml), the organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 3.62g of crude yellow/brown oil. This was flash chromatographed, eluting with petrol:EtOAc (1:1) to give recovered starting material (1.88g, 72%) and the β-hydroxyphosphonate **(436)** (0.04g, 2%) as a colourless oil.

***Attempted preparation of 3(S)-N,N-dibenzylamino-1-(diethylphosphonate)-2(R,S)-(O-methyl leucinyl)butane* (434) *from the γ-chlorophosphonate* (444).**

**Method A**

To a suspension of Leu-OMe.HCl (0.48g, 2.64mmol) in THF (4ml) was added triethylamine (0.334ml, 2.4mmol), the reaction was stirred for 30 minutes before being filtered and added to the γ-chlorophosphonate **(444)** (0.646g, 1.53mmol) in THF (4ml) at 0°C. After 5 hours no reaction occurred, the reaction was allowed to attain room temperature and left for 6 days, no reaction had occurred. The reaction was abandoned.

**Method B**

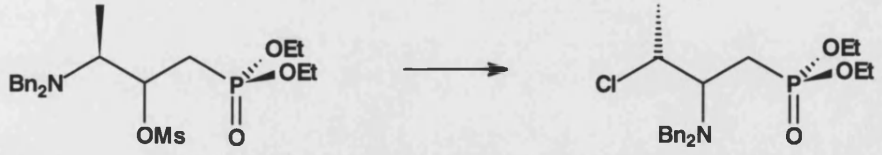
To a freshly prepared solution of Leu-OMe (0.047g, 0.32mmol) in THF (1ml) was added NaOAc (0.013g, 0.16mmol) and the γ-chlorophosphonate **(444)** (0.086g, 0.2mmol) in THF (1ml). The reaction was stirred at room temperature for 3 days no reaction had occurred. The reaction was abandoned.

**Method C**

To a freshly prepared solution of Leu-OMe (0.047g, 0.32mmol) in THF (1ml) was added the γ-chlorophosphonate **(444)** (0.086g, 0.2mmol) in DMF (1ml). The reaction was stirred for 3 days no reaction had occurred. It was then refluxed for 2 hours, no change occurred so the reaction was abandoned.

***Attempted preparation of (2R,3S,2'S) and (2S,3S,2'S)-3-N,N-dibenzylamino-1-diethylphosphonate-2-(O-methyl leucinyl)butane* (434) *from the methanesulfonate* (448).**

To a solution of the methanesulfonate **(448)** (0.078g, 0.16mmol) in THF (20ml) was added Leu-OMe.HCl (0.035g, 0.19mmol). The reaction was stirred at room temperature for 1 hour. T.l.c. showed that all the starting material had been consumed. The reaction mixture was washed with brine (20ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the γ-chlorophosphonate **(444)** (0.033g, 50%).




**(448)** **(444)**

***(2S,3S)- and (2R,3S)-3-N,N-Dibenzylamino-1-(diethylphosphonate)-2-hydroxy-butane* (436).**

To a cooled solution (0°C) of the β-ketophosphonate **(432)** (2.43g, 6.0mmol) in EtOH (30ml) was added $NaBH_4$ (0.062g, 1.65mmol). The reaction was stirred for 10 minutes before being allowed to attain room temperature. After 6 hours some starting material still remained, some more $NaBH_4$ (0.01g) was added and the reaction was left to stir overnight after which time all the starting material had been consumed. The reaction mixture was diluted with EtOAc (100ml), washed with water (100ml), brine (100ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the β-hydroxyphosphonate **(436)** (2.3g, 95%) as a colourless oil.


**(432)** **(436)**

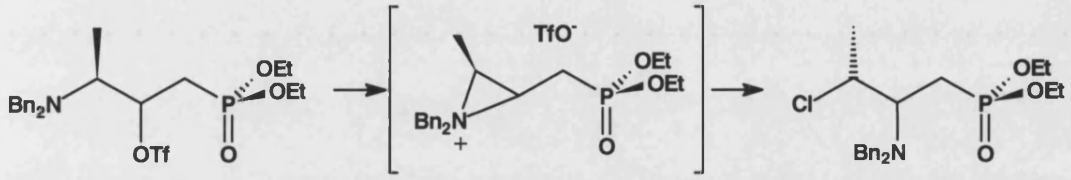
***Attempted preparation of (2S,3S)- and (2R,3S)-3-N,N-dibenzylamino-1-(diethylphosphonate)-2-(trifluoromethanesulfonyloxy)butane* (441).**

To a cooled solution (-20°C) of the β-hydroxyphosphonate **(441)** (0.83g, 2.052mmol) in DCM (20ml) was added pyridine (0.191ml, 2.36mmol), the reaction was stirred for 5 minutes before the addition of triflic anhydride (0.38ml, 2.26mmol). The reaction was then allowed to stir at -20°C for 5 minutes before being allowed to attain room temperature and stirred for 1 hour. The reaction mixture was diluted with ether (30ml), washed with water (30ml), brine (30ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 1.02g of a deep red viscous oil. This was flash chromatographed, eluting with petrol:EtOAc (1:1) to give the rearranged γ-chlorophosphonate **(444)** (0.7g, 64%):

***(2S,3R)- and (2R,3R)-2-N,N-Dibenzylamino-3-chloro-1-(diethylphosphonate) butane* (444).**

(2R,3*R*)- and (2*S*,3*R*)-2-*N*,*N*-Dibenzylamino-3-chloro-1-(diethylphosphonate) butane **(444)** were isolated as a colourless oil, $R_f$ [petrol:EtOAc (1:1)]; 0.38;

$\nu_{max}/cm^{-1}$ 2980, 1737, 1603, 1494, $1453_{\delta\ as}$ (Me), $1370_{\delta\ s}$ (Me), $1244_{s}$ (P=O), $1027_{s}$ (P-O); $\delta_H$(270 MHz, $CDCl_3$) 1.32 (3H, t, *J* 6.9, $CH_2CH_3$), 1.33 (3H, t, *J* 6.9, $CH_2CH_3$), 1.43 (3H, d, *J* 6.9, CH*Me*), 2.17-2.41 (2H, m, $CH_2P$), 3.21 (1H, dddd, *J* 12.7, 9.8, 2.9 and 2.9, C*H*$NBn_2$), 3.29 (2H, d, *J* 13.5, $CH_2N$), 4.10 (2H, d, *J* 13.5, $CH_2N$), 4.00-4.20 (4H, dq, *J* 7.1 and 7.1, $POCH_2$), 4.33-4.40 (1H, m, CHCl) and 7.22-7.8 (10H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 16.3 and 16.4 ($CH_2CH_3$), 22.9 (CH*Me*), 21.1 and 23.1 ($CH_2P$), 55.5 ($CH_2N$), 56.6 (CHN), 61.6 and 61.7 ($CH_2CH_3$), 62.4 (CHCl), 127.1 (*p*-phenyl), 128.1 (*o*-phenyl), 129.3 (*m*-phenyl) and 139.3 (phenyl); m/z (C.I.) 424 ($M^+$+1, 100%), 388 (45, $M^+$-Cl); [Found : ($M^+$+1) 424.1815. $C_{22}H_{32}NO_3ClP$ requires 424.1808].




**(441)** **(442)** **(444)**

***Attempted preparation of (2S,3S)- and (2R,3S)-3-N,N-dibenzylamino-1-(diethylphosphonate)-2-(methanesulfonyloxy)butane* (448).**

To a cooled solution (0°C) of the β-hydroxyphosphonate **(436)** (0.094g, 0.23mmol) in DCM (2ml) was added triethylamine (0.125ml, 1.54mmol) and methanesulfonyl chloride (0.319g, 1.67mmol) over 5 minutes. The reaction was stirred for 30 minutes at 0°C. The reaction mixture was diluted with DCM (10ml), washed with iced water (10ml), 0.5M HCl (30ml), sat. solution of $NaHCO_3$ (10ml), brine (10ml), dried ($MgSO_4$) and concentrated *in vacuo* to give methanesulfonate as a colourless oil **(448)** (0.088g, 80%), this was used without purification; crude $\delta_H$(60 MHz, $CDCl_3$) 1.1-1.5 [9H, m, Me and $P(OCH_2CH_3)_2$], 2.0-2.4 (2H, m, $CH_2PO$), 2.9 (3H, s, $SO_2CH_3$), 3.0-3.5 (2H, m, C*H*Me and CHO), 3.6-4.2 [6H, m, $(PhCH_2)_2N$ and $PO(CH_2CH_3)_2$] and 7.2-7.4 (10H, m, phenyl).


**(436)** **(448)**

***(2R,3S)- and (2S,3S)-3-N,N-dibenzylamino-2-hydroxy-1-(4'-methoxyphenylmethyl-thio) butane* (455a) *and* (455b).**

To a stirred solution of the epoxides (1.15g, 4.3mmol) in anhydrous methanol (20ml) was added triphenylmethylthiol (0.6ml, 4.3mmol) and triethylamine (0.6ml, 4.3mmol). The reaction was then refluxed for 3 hours. The reaction mixture was filtered to remove the excess triphenylmethylthiol. The reaction mixture was concentrated *in vacuo* to give 2.05g of crude material, which was flash chromatographed, eluting with cyclohexane:EtOAc (92:8) to give the desired products **(455a)** (1.16g, 64%) and **(455b)** (0.61g, 35%):

***(2R,3S)-3-N,N-dibenzylamino-2-hydroxy-1-(4'-methoxyphenylmethylthio) butane***

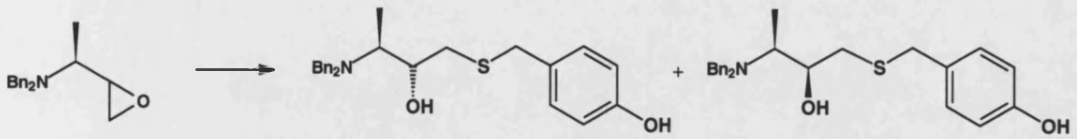
**(455a).**

(2*R*,3*S*)-3-*N*,*N*-dibenzylamino-2-hydroxy-1-(4'-methoxyphenylmethylthio) butane **(455a)** was isolated as a colourless oil; $R_f$[petrol:EtOAc (90:10)] 0.21; (Found C, 73.50; H, 7.60; N, 3.30. calc. for $C_{26}H_{31}NO_2S$: C, 74.07; H, 7.41; N, 3.32%); $\nu_{max}/cm^{-1}$ $3348_s$ (OH), 1608, 1511, $1455_{\delta\ as}$ (Me), $1248_s$ (C-O), $1033_s$ ($SCH_2$) and $700_s$ (C-S); $\delta_H$(270 MHz, $CDCl_3$) 1.14 (3H, d, *J* 6.8, Me), 2.18 (1H, dd, *J* 14.1 and 10.1, S*CH*$_2$CHO), 2.60 (1H, qd, *J* 7.0 and 4.0, C*H*Me), 3.03 (1H, dd, *J* 10.1 and 2.4, S*CH*$_2$CHO), 3.38 (2H, d, *J* 13.7, $CH_2N$), 3.61 (2H, s, $SCH_2Ar$), 3.62 (2H, d, *J* 13.7, $CH_2N$), 3.73 (3H, s, OMe), 3.60-3.75 (2H, m, C*HOH*), 6.70-6.80 (2H, m, *Ar*OMe), 7.10-7.20 (2H, m, *Ar*OMe) and 7.20-7.30 (10H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.1 (Me), 35.1 ($CH_2SCH_2Ar$), 37.5 ($CH_2S$), 54.3 ($CH_2N$), 54.8 (OMe), 57.3 (N*C*HMe), 70.5 (CHOH), 113.9 (*o*-*Ar*OMe), 126.0-130.0 (phenyl and

*Ar*OMe), 139.8 (phenyl) and 158.7 (*Ar*OMe); m/z (E.I. 70 eV) 224 (5%, $Bn_2NCHMe^+$); (C.I) 422 ($M^+$+1, 80), 224 (100, $Bn_2NCHMe^+$).

***(2S,3S)-3-N,N-Dibenzylamino-2-hydroxy-1-(4'-methoxyphenylmethylthio) butane* (455b).**

(2*S*,3*S*)-3-*N*,*N*-Dibenzylamino-2-hydroxy-1-(4'-methoxyphenylmethylthio) butane **(455b)** was isolated as a colourless oil; $R_f$[petrol:EtOAc (90:10)] 0.13; (Found C, 73.50; H, 7.60; N, 3.30. calc. for $C_{26}H_{31}NO_2S$: C, 74.07; H, 7.41; N, 3.32%); $\nu_{max}/cm^{-1}$ $3348_s$ (OH), 1608, 1511, $1455_{\delta\ as}$ (Me), $1248_s$ (C-O), $1033_s$ ($SCH_2$) and $700_s$ (C-S); $\delta_H$(270 MHz, $CDCl_3$) 1.18 (3H, d, *J* 6.8, Me), 2.65-2.74 (1H, m, $SCH_2CHO$), 2.75-2.98 (2H, m, *CH*Me and $SCH_2CHO$), 3.26 (2H, d, *J* 13.7, $CH_2N$), 3.61 (2H, s, $SCH_2Ar$), 3.70-3.82 (2H, m, *CHOH*), 3.70 (2H, d, *J* 13.7, $CH_2N$), 3.78 (3H, s, OMe), 6.80-6.88 (2H, m, *Ar*OMe), 7.10-7.20 (2H, m, *Ar*OMe) and 7.20-7.35 (10H, m, phenyl); $\delta_C$ (67.8 MHz, $CDCl_3$) 14.2 (Me), 34.7 ($CH_2SCH_2Ar$), 36.6 ($CH_2S$), 54.1 ($CH_2N$), 55.3 (OMe), 56.9 (*N*CHMe), 71.4 (CHOH), 113.9 (*o*-*Ar*OMe), 114.0 (*o*-*Ar*OMe), 127.1 (*p*-phenyl), 127.4 (phenyl), 128.3 (phenyl), 128.5 (phenyl), 129.0 (phenyl), 129.2 (*Ar*OMe), 129.9 (*Ar*OMe), 130.0 (*Ar*OMe), 130.1 (*Ar*OMe), 138 (phenyl-$CH_2O$) and 139.39 (phenyl-$CH_2N$), 139.4 (phenyl) and 159.0 (*Ar*OMe); m/z (E.I. 70 eV) 224 (5%, $Bn_2NCHMe^+$); (C.I) 422 ($M^+$+1, 80), 224 (100, $Bn_2NCHMe^+$).




**(356)** and **(357)** **(455a)** **(455b)**

***Attempted preparation of (2R,3S) and (2S,3S)-3-N,N-dibenzylamino-1-(4'-methoxyphenylmethylthio)-2-(trifluoromethanesulfonyloxy)butane* (451a) *and* (451b).**

To a cooled solution (-20°C) of the protected thiolalcohol (**450**) (0.47g, 1.15mmol) in DCM (6ml) was added pyridine (0.103ml, 1.27mmol). The reaction was stirred for 5 minutes before the addition of triflic anhydride (0.213ml, 1.27mmol). It was then allowed to stir at -20°C for 5 minutes before being warmed to room temperature and stirred for a further 1 hour. The reaction mixture was diluted with DCM (5ml), washed with 1% HCl (10ml), brine (10ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 0.62g of a crude red oil. T.l.c. showed a huge number of spots and the crude mixture was not purified.

***Attempted preparation of (2R,3S) and (2S,3S)-3-N,N-dibenzylamino-1-(4'-methoxyphenylmethylthio)-2-(p-toluenesulfonyloxy)butane* (454a) *and* (454b).**

**Method A**

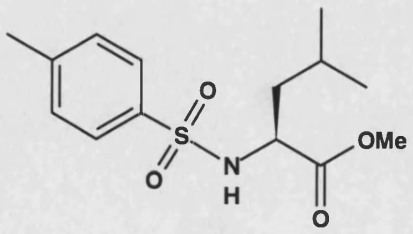
To a cooled solution (0°C) of the alcohol (**450**) (0.4g, 0.95mmol) in DCM (10ml) was added pyridine (0.076ml, 0.94mmol) and *p*-toluenesulfonyl chloride (0.181g, 1.0mmol) in small portions with constant stirring. The reaction was stirred at 0°C for 6 hours, no change was observed by t.l.c., so the reaction was allowed to attain room temperature and stirred for a further 3 days. No change was observed, the reaction mixture was washed with 2M HCl (10ml), the acid phase back extracted with DCM (2 x 10ml), the organics combined, dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed, eluting with petrol:EtOAc (87.5:12.5) to give the recovered starting material (0.306g, 78%).

**Method B**

To a cooled solution (-20°C) of the alcohol **(450)** (0.306g, 0.73mmol) in THF (20ml) was added NaH (0.024g, 0.80mmol). After 10 minutes *p*-toluenesulfonyl chloride (0.166g, 0.87mmol) was added, after 10 minutes t.l.c. showed that some of the starting material had been converted to a new compound. After 2 hours Leu-OMe.HCl (0.20g, 1.10mmol) and the reaction mixture was left to stir for 3 hours. The reaction mixture was washed with 2M HCl (20ml), the acid phase back extracted with EtOAc (2 x 20ml), the organics combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 0.42g of crude material. This was flash chromatographed, eluting with petrol:EtOAc (90:10) to give the recovered starting material (0.1g, 33%) and Tos-Leu-OMe **(456)** as a colourless oil (0.069g, 32%); $R_f$[petrol:EtOAc (90:10)] 0.61.

***N-Toluenesulfonate-Leu-OMe* (456).**

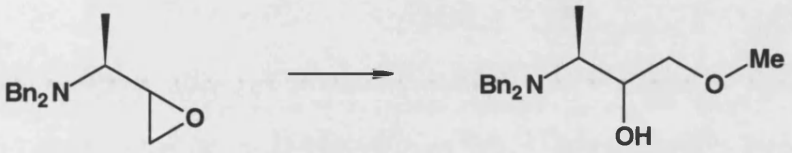
*N*-Toluenesulfonate-Leu-OMe **(456)** was isolated as a colourless oil; (Found C, 56.50; H, 7.17; N, 4.38. calc. for $C_{19}H_{21}NO_4S$: C, 56.17; H, 7.07; N, 4.68%); $\nu_{max}/cm^{-1}$ $3273_s$ (NH), $1741_s$ (C=O), $1337_s$ (O=S-N) and 1163; $\delta_H$(270 MHz, $CDCl_3$) 0.87 (3H, d, *J* 6.4, Me), 0.90 (3H, d, *J* 6.4, Me), 1.45-1.50 (2H, m, $CH_2CHMe_2$), 1.74-1.81 (1H, m, $CHMe_2$), 2.42 (3H, s, phenyl-*Me*) 3.43 (3H, s, OMe), 5.11-5.15 (1H, m, N*H*-$SO_2$), 7.29 (2H, d, *J* 8.3, phthaloyl) and 7.72 (2H, d, *J* 8.3, phthaloyl); m/z (E.I. 70 eV) 240 (100%, $M^+$-$CO_2Me$); (C.I) 300 ($M^+$+1, 100), 240 (90, $M^+$-$CO_2Me$).




**(456)**

***Failed preparation of (2R,3S)- and (2S,3S)-3-N,N-dibenzylamino-2-hydroxybutyl-oxymethyl ether* (491).**

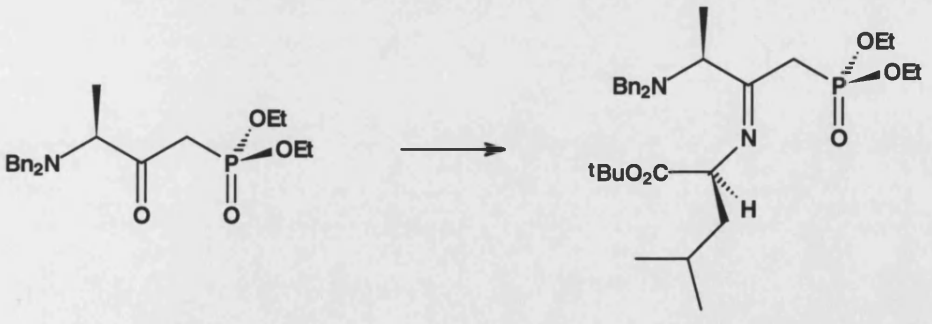
To a solution of NaOMe (0.045g, 0.83mmol) in DMF (2ml) was added epoxides (**356**,**357**:**366**, 1:1) (0.074g, 0.28mmol). The reaction mixture was heated to 100°C for 10 hour, cooled, and partitioned between water (5ml) and ether (5ml). The aqueous layer was extracted with ether (2 x 5ml), the organics combined, washed with water (5ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was flash chromatographed on silica, eluting with petrol:EtOAc (80:20) to give only the starting material **(491)** (0.05g, 68%).




**(356) and (357)** **(491)**

***Attempted preparation of the imine* (492) *using Leu-O$^t$Bu***

To the β-ketophosphonate **(432)** (0.613g, 1.52mmol) in toluene (50ml) was added Leu-O$^t$Bu.HCl (1.04g, 4.64mmol) and $TiCl_4$ (0.144g, 0.76mmol). The reaction was refluxed under an inert atmosphere of nitrogen for 2 days. After this time no reaction had occurred and the reaction was abandoned.




**(432)** **(492)**

***Attempted preparation of (2S,3S)- and (2R,3S)-3-N,N-dibenzylamino-1-(diethylphosphonate)-2-(toluenesulfonyloxy)butane* (493).**

To a cooled solution (0°C) of the β-hydroxyphosphonate **(436)** (0.52g, 1.28mmol) in DCM (20ml) was added pyridine (0.125ml, 1.54mmol), *p*-toluenesulfonyl chloride (0.319g, 1.67mmol) in small portions with constant stirring. The reaction was stirred at 0°C for 1 day, no change was observed by t.l.c., so the reaction was allowed to attain room temperature and stirred for a further 3 days. The reaction mixture was then refluxed for 4 hours after which time no reaction had occurred. The reaction mixture was washed with 2M HCl (30ml), the acid phase back extracted with DCM (2 x 100ml), the organics combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 0.6g of crude material. This was flash chromatographed, eluting with petrol:EtOAc (1:1) to give the recovered starting material.


**(436)** **(493)**

## REFERENCES


1. The Organic Chemistry of Drug Design and Drug Action, R.B. Silverman, Academic Press Inc., London, 1992, Ch 4 and 5.
2. J.B.S. Haldane, Enzymes, Longmans, Green, London, 1930 (reprinted in 1965) by MIT Press, Cambridge, Massachusetts.
3. H. Eyning, *J. Phys. Chem.*, 1935, **3**, 107.
4. L. Pauling, *Chem. Eng. News*, 1946, **24**, 1375; *Am. Sci.*, 1948, **36**, 51.
5. G.J. Moore, *Trends in Pharmaco. Science*, 1994, **15**, 124.
6. IUPAC-IUB Commission on Biochemical Nomenclautre, 1970, **9**, 3471; G.E. Schulz, R.H. Schirmer, *Principles of Protein structure*, C.R. Cantor, Ed. Springer-Verlog, N.Y. 1979, p. 18.
7. G. Némethy, H.A. Schreaga, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 320.
8. a) A.F. Spatola, *Chem. Biochem., Amino Acids, Pept. Proteins*, 1983, **7**, 267;
   b) J.S. Davies, *Amino Acids, Peptides and Proteins*, The Royal Society of Chemistry, Cambridge, Ch. 3, 1983-1992;
   c) J. Gante, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1699;
   d) R.A. Wiley and R.H. Rich, *Med. Res. Rev.*, 1993, **13**, 327;
   e) Drug Discovery Technologies, C.R. Clark and W.H. Moos, Ellis Horwood Ltd. Chichester, 1990, Ch 5.
   f) Peptidomimetics Derived from Natural Products: S. Thaisrivongs, *Annu. Rep. Med. Chem.*, 1994, **9**, 133.
9. Renin : W.J. Greenlee, *Med. Res. Rev.*, 1990, **10**, 173; HIV proteinase : P.S. Anderson, G.L. Kenyou and G.R. Marshall, *Perspectives in Drug Discovery and Design*, 1993, **1**, 1.
10. S.A. Bernhard and L.E. Orgel, *Science*, 1959, **130**, 625.

**Reduced amide isosteres:**

11. ACE inhibitors: - M. Szelke, B. Leckie, A. Hallett, D.M. Jones, J. Sueiras,

B. Atrash, A.F. Lever, *Nature*, 1982, **299**, 555.

Renin inhibitors: - M. Szelke, D.M. Jones, B. Atrash, A. Hallett and B.J. Leckie, *Peptides, Structure and Function*: Proceedings of the 8th American Peptide Symposium. V.J. Hruby and D.H. Rich, Eds., Pierce Chemical Co., Rochford, IL, 1983, p. 579.

12. M.J. Parry, A.B. Russell and M. Szelke, in *Chemistry and Biology of Peptides*, J. Meinhofer, Ed., Ann Arbos Science Publishers, Ann, Arbor, 1972, p. 541.

13. B. Leckie, M. Szelke, A. Hallett, M. Hughes, A.F. Lever, G. McIntyre, J.J. Morton and M. Tree, *Clin. Exp. Hypertension - Theory Pract.*, 1983, **A5**, 1221.

14. B.J. Leckie, M. Szelke, B. Atrash, S.R. Beattic, A. Hallett, D.M. Jones, G.D. McIntyre, J. Suerias and D.J. Webb, *Biochem. Soc. Trans.*, 1985, **13**, 1029.

15. J. Martinez, J-P. Bali, M. Rodriguez, B. Castro, R. Magous, J. Laur and, M-F Lignon, *J. Med. Chem.*, 1985, **28**, 1874.

16. M. Rodriguez, J-P. Bali, R. Magous, B. Castro and J. Martinez, *Int. J. Pept. Protein Res.*, 1986, **27**, 293; J. Martinez, M. Rodriguez, J-P. Bali and J. Laur, *ibid.*, 1986, **28**, 529; *J. Med. Chem.*, 1986, **29**, 2101.

17. A. Amuelas, M. Rodriguez, A. Heitz, B. Castro and J. Martinez, *Int. J. Pept. Protein Res.*, 1987, **30**, 596.

18. J.J. Plattner, J. Greer, A.K.L. Fung, H. Stein, H.D. Kleinert, H.L. Sharn, J.R. Smital and T.J. Perun, *Biochem. Biophys. Res. Commun.*, 1986, **139**, 982.

19. P. van der Elst, M. Elseviers, E. de Cock, M. van Marseinille, D. Tourwe and G. van Binst, *Int. J. Pept. Protein Res.*, 1986, **27**, 633.

20. M. Lebl, E.E. Sugg, G. van Binst, P. van der Elst, D. Tourwe, J. Slaninova and V.J. Hruby, *Int. J. Pept. Protein Res.*, 1987, **30**, 318.

21. Y. Sasaki, W.A. Murphy, M.L. Heiman, V.A. Iance and D.H. Coy, *J. Med. Chem.*, 1987, **30**, 1162; D.H. Coy, S.J. Hocart and Y. Sasaki, *Tetrahedron*, 1988, **44**, 835; S.J. Hocart and D.H. Coy, *Innovation and Perspectives in Solid*

*Phase Synthesis,* Peptides, Polypeptides and Oligonucleotides, Macro-organic Reagents and Catalysts, 1990, Ed. R. Epton, Coll. Papers; 1st Int. Symposium, 1989, 413; other examples; W.M. Kazierski, R.D. Ferguson, R.J. Knapp, G.K. Liu, H.I. Yamamura and V.J. Hruby, *Int. J. Pept. Protein Res.*, 1992, **39**, 401; N.G.J. De Lact, P. Verheyden, B. Velkeniers, E.L. Hooghepeters, C. Burns, D. Tourwe and G. van Binst, *Peptide Res.*, 1993, **6**, 24.

22. S.J. Hocart, W.A. Murphy and D.H. Coy, *J. Med. Chem.*, 1990, **33**, 1954.

23. J.M. Qian, D.H. Coy, N.Y. Jiang, J.D. Gardner and R.T. Jensen, *J. Biol. Chem.*, 1989, **264**, 16667; S. Zacharia, W.J. Rossowski, N.Y. Jiang, P. Hibas, A. Ertan and D.H. Coy, *Eur. J. Pharm.*, 1991, **203**, 353.

24. B.M. Haffar, S.J. Hocart, D.H. Coy, S. Mantley, H.C.V. Chiang and R.T. Jensen, *J. Biol. Chem.*, 1991, **266**, 316.

25. M. Rodriguez, M-F. Lignon, M-C Galas, P. Fulcrand, C. Mander, A. Aumelus, J. Laur and J. Martinez, *J. Med. Chem.*, 1987, **30**, 1366; C.I. Fincham, M. Higginbottom, D.R. Hill, D.C. Honvell, J.C. O'Toole, G.S. Ratcliffe, D.C. Rees and E. Roberts, *J. Med. Chem.*, 1992, **35**, 1472.

26. H. Oyamada and M. Ueli, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 267.

27. T.K. Sawyer, D.T. Pals, B. Mao, L.C. Maggiora, D.J. Staples, A.E. de Vaux, H.J. Schostarez, J.H. Kinner and C.W. Smith, *Tetrahedron,* 1988, **44**, 661.

28. S.J. Hocart, M.K. Nekola and D.H. Coy, *J. Med. Chem.*, 1988, **31**, 1820.

29 a) D.H. Coy, P. Heinz-Erian, N-Y Jiang, Y. Sasaki, J.Taylor, J-P. Moreau, W.T. Wolfrey, J.D. Gardner and R.T. Jensen, *J. Biol. Chem.*, 1988, **263**, 5056,

b) S. Mahmoud, E. Palaszysnki, G. Fiskum, D.H. Coy, T.W. Moody, *Life Sciences,* 1989, **44**, 367;

c) R.Z. Cai, S. Radulovic, J. Pinski, A. Nagy, T.W. Redding, D.B. Olsen and A.V. Schally, *Peptides,* 1992, **13**, 267;

d) R. De Castiglione, L. Gozzini, M. Galantino, F. Corradi, M. Ciomei, F. Roletto and F. Bertolero, *Farmaco,* 1992, **47**, 855.

30. F.S. Guziec and L.M. Wasmund, *Tetrahedron Lett.*, 1990, **31**, 23.

31. A. Giannis and K. Sandhoff, *Angew. Chem. Int. Ed. Engl.*, 1989, **28**, 218.

32. A. Scarso, J. Degelaen, R. Viville, E. DeCock, M. van Marsenille, L. van der Auwera, D. Tourwe and G. van Binst, *Bull. Soc. Chim. Belg.*, 1991, **100**, 381.

33. J.A. Straub, A. Akiyama, P. Parmar and G.F. Musso, *J. Chromatogr.*, 1994, **679**, 85.

34. D.H. Coy and Y. Sasaki, *Peptides*, 1987, **8**, 119.

35. P.T. Ho, D. Chang, J.W.X. Zhong and G.F. Musso, *Peptide Res.*, 1993, **6**, 10.

36. D. Lugrin, F. Vecchini, S. Doulut, M. Rodriguez, J. Martinez and P. Kitabgi, *Eur. J. Pharmaco.*, 1991, **205**, 191; S. Doulut, M. Rodriguez, D. Lugrin, F. Vecchini, P. Kitabgi, A. Aumelas and J. Martinez, *Peptide Res.*, 1992, **5**, 30.

37. T.E. Christos, A. Arvanitis, G.A. Cain, A.L. Johnson, R.S. Pottorf, S.W. Tam and W.K. Schmidt, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 1035.

38. D. Jukic, M. Mayer, P. Schmitt, G. Drapeau, D. Regoli and R. Michelot, *Eur. J. Med. Chem.*, 1991, **26**, 921.

39. S.L. Harbenson, S.A. Shatzer, T.B. Le and S.H. Buck, *J. Med. Chem.*, 1992, **35**, 3949.

40. N.G.J. DeLaet, P.M.F. Vereyden, D. Tourwe, G. van Binst, P. Davis and T.F. Burks, *Biopolymers*, 1992, **32**, 957.

41. S.H. Nakagawa, N.L. Johansen, K. Madsen, T.W. Schwartz and H.S. Tager, *Int. J. Pept. Protein Res.*, 1993, **42**, 578.

42. P.W. Schiller, G. Weltrowska, T. M-D. Nguyen, B.C. Wilkes, N.N. Chung and C. Lemieux, *J. Med. Chem.*, 1993, **36**, 3182; and references therein.

43. S. Salvadori, R. Guerrini, P.A. Borea and T. Tomatis, *Int. J. Pept. Protein Res.*, 1992, **40**, 437; S. Miertus, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 2105.

44. M. Miller, J. Schneider, B.K. Sathyanarayana, M.V. Toth, G.R. Marshall, L. Clawson, L. Selk, S.B.H. Kent and A. Wlodawer, *Science*, 1989, **246**, 1149.

45. P.M.D. Fitzgerald, B.M. McKeever, J.F. van Middlesworth and J.P. Springer, A. In Kumar (Ed), *Advances in Molecular Biology and Targeted Treatment for AIDS,* Plenum Press, New York, 1991, p. 245-249.

46. M. Sakurai, M. Sugano, H. Handa, T. Komai, Y. Ryuicki, T. Nishigaki and Y. Yabe, *Chem. Pharm. Bull.*, 1993, **41**, 1369.

47. M.L. Nedved and G.R. Moe, *Nucleic Acids Res.*, 1994, **22**, 4705.

48. A. Geyer, G. Muller, and H. Kessler, *J. Am. Chem. Soc.*, 1994, **116**, 7735.

49. A.F. Spatola and S.G. Ma, *Int. J. Pept. Protein Res.*, 1993, **41**, 204.

50. P. Dauber-Osguthorpe, M.M. Campbell and D.J. Osguthorpe, *Int. J. Pept. Protein Res.*, 1991, **4**, 357; P. Dauber-Osguthorpe, D.K. Jones, M.M. Campbell, G. Semple and D.J. Osguthorpe, *Tetrahedron Lett.*, 1990, **31**, 917.

51. M. Cushman, Y-I. Oh, T.D. Copeland, S. Oroszlan and S.W. Snyder, *J. Org. Chem.*, 1991, **56**, 4161.

52. M. Veki, K. Miyamoto and H. Oyamada, Proceedings of the 21st European Peptide Symposium, 1990, p370.

53. J-P. Salvi, N. Walchshofer and J. Paris, *Tetrahedron Lett.*, 1994, **35**, 1181.

54. K.A. Newlander, J.F. Callahan, M.L. Moore, T.A. Tomaszek and W.F. Hoffman, *J. Med. Chem.*, 1993, **36**, 2321.

55. R.T. Shuman, R.B. Rothenberger, C.S. Campbell, G.F. Smith, D.S. Gifford-Moore and P.D. Gescellchen, *J. Med. Chem,* 1993, **36**, 314.

56. R.D. Nicolaides, F.J. Tinney, J.S. Kaltenbronn, J.T. Repine, D.A. DeJohn, E.A. Lunney, W.H. Roark, I.G. Marriott, R.E. Davis and R.E. Voigtman, *J. Med. Chem.*, 1986, **29**, 959.

**Hydroxyethylene isosteres**

57. R.J. Workman and D.W. Burkitt, *Arch. Biochem. Biophys.*, 1979, **194**, 157.

58. F. Cumin, G. Evin, J.A. Fehrentz, R. Seyer, B. Castro, J. Menard and P. Corvol, *J. Biol. Chem.*, 1985, **260**, 9154.

59. D.H. Rich, *J. Med. Chem.*, 1985, **28**, 263.

60. M. Szelke, D.M. Jones and A. Hallett, European Patent Application, 1982, EP 45,665.

61. M.W. Holloday, D.H. Rich, *Tetrahedron Lett.*, 1983, **24,** 4401.

62. B.E. Evans, K.E. Rittle, C.F. Homnick, J.P. Springer, J. Hirshfield and D.F. Veber, *J. Org. Chem.*, 1985, **50,** 4615-25.

63. P. Bühlmayer, A. Caselli, W. Fuhrer, R. Göschke, V. Rasetti, H. Rüeger, J.L. Stanton, L. Crisione and J.M. Wood, *J. Med. Chem.*, 1988, **31,** 1839.

64. E.J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, 1965, **87,** 1353.

65. S. Thaisrivongs, D.T. Pals, W.M. Kati, S.R. Turner, L.M. Thomasco and W. Watt, *J. Med. Chem.*, 1986, **29,** 2080; *ibid,* 2088.

66. R. Day and E. Haber, *Handbook for Hypertension,* Vol. 8, Eds. A. Zanchetti and R.C. Tarazi, Elsevier Science Publications, B.V., Amsterdam, 1986, p. 315.

67. S. Thaisrivongs, D.T. Pals, L.T. Kroll, S.R. Turner and F-S Han, *J. Med. Chem.*, 1987, **30,** 976.

68. S.E. deLaszlo, Merck Sharp and Dohme Research Laboratories, Rahway, NJ, unpublished results.

69. H. Rüeger, P. Bühlmayer, W. Fuhrer, R. Göschke, V. Rasetti, J. Stanton, and J. Wood, Abstracts and slides of the 21st National Medical Chemistry Symposium, Minneapolis, MN, June 19-23, 1988, American Chemical Society, p. 69.

70. R. Metternich and W. Lüdi, *Tetrahedron Lett.*, 1988, **29,** 3923.

71. R.H. Bradbury, J.S. Major, A.A. Oldham, J.E. Rivetti, D.A. Roberts, A.M. Slater, D. Timms and D. Waterson, *J. Med. Chem.*, 1990, **33,** 2335.

72. M. Shiozaki and Y. Kobayashi, *Tetrahedron,* 1991, **47,** 2785; M. Shiozaki, T. Hata and Y. Furukawa, *Tetrahedron Lett.*, 1989, **30,** 3669.

73. S.E. de Laszlo, B.L. Bush, J.J. Doyle, W.J. Greenlee, D.G. Hangauer, T.A. Halgren, R.J. Lynch, T.W. Schorn and P.K. Siegl, *J. Med. Chem.*, 1992, **35,** 833.

74. S. Atsuumi, M. Nakano, Y. Koike, S. Tanaka, K. Matsuyama, M. Nakano and H. Morishima, *Chem. Pharm. Bull.*, 1992, **40**, 364.

75. W.R. Baker and J.K. Pratt, *Tetrahedron*, 1993,**49**, 8739; Abstracts of papers of the *Amer. Chem. Soc.*, 1992, **204**, 42.

76. S. Hannessian and S. Raghavan, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1697.

77. D.J. Kempf, *J. Org. Chem.*, 1986, **51**, 3922.

78. J.V.N.V. Prasad and D.H. Rich, *Tetrahedron Lett.*, 1990, **31**, 1803; *ibid*, 1991, **32**, 5857.

79. R. Hanko, K. Rabe, R. Dally and D. Hoppe, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1690.

80. F. D'Aniello, S. Géhanne and M. Taddei, *Tetrahedron Lett.*, 1992, **33**, 5621; F. D'Aniello, D. Mattii and M. Taddei, *Synlett.*, 1993, 119; F. D'Aniello, A.Mann, D. Mattii and M. Taddei, *J. Org. Chem.*, 1994, **59**, 3762.

81. S-I. Kiyooka, Y. Shiomi, H. Kira, Y. Kaneko and S. Tanimori, *J. Org. Chem.*, 1994, **59**, 1958.

82. P. Ciapetti, M. Taddei and P. Ulivi, *Tetrahedron Lett.*, 1994, **35**, 3183.

83. M.W. Holladay, F.G. Salituro and D.H. Rich, *J. Med. Chem.*, 1987, **30**, 374.

84. P. Herold, R. Duthaler, G. Rihs and C. Angst, *J. Org. Chem.*, 1989, **54**, 1178.

85. R.H. Bradbury, J.M. Revill, J.E. Rivett and D. Waterson, *Tetrahedron Lett.*, 1989, **30** 3845.

86. P.G.M. Wuts, A.R. Ritter and L.E. Pruitt, *J. Org. Chem.*, 1992, **57**, 6696; P.G.M. Wuts, S.R. Putt and A.R. Ritter, *ibid.*, 1988, **53**, 4502; H.G. Chen, T.K. Sawyer and P.G.M. Wuts, *Acta Pharmacologica Sinica*, 1994, **15**, 33.

87. D.M. Jones, B. Nilsson and M. Szelke, *J. Org. Chem.*, 1993, **58**, 2286.

88. H. Eckert, M. Listl and I. Ugi, *Angew. Chem.*, 1978, **90**, 388.

89. A.M. Diederich and D.M. Ryckman, *Tetrahedron Lett.*, 1993, **34**, 6169; Abstracts of papers of the *Amer. Chem. Soc.*, 1994, **208**, 101.

90. P.K. Chakravarty, S.E. de Laszlo, C.S. Sarnella, J.P. Springer and P.F. Schuda, *Tetrahedron Lett.*, 1989, **30**, 415.

91. A. Basha, M. Lipton, S.M. Weinreb, *Tetrahedron Lett.*, 1977, **18**, 4171.

92. M.A. Poss and J.A. Reid, *Tetrahedron Lett.*, 1992, **33**, 1411; M.A. Poss and J. Reid, Abstracts of papers of the *Amer. Chem. Soc.*, 1990, **199**, 60.

93. D.J. Plata, M.R. Leanna and H.E. Morton, *Tetrahedron Lett.*, 1991, **32**, 3623.

94. A. Dondoni and D. Perrone, *Tetrahedron Lett.*, 1992, **33**, 7259.

95. a) S.H. Rosenberg, S.A. Boyd and R.A. Mantei, *Tetrahedron Lett.*, 1991, **32**, 6507

b) S.A. Boyd, R.A. Mantei, C-N. Hsiao and W.R. Baker, *J. Org. Chem.*, 1991, **56**, 438.

96. S. Kano, T. Yokomatsu and S. Shibuya, *Tetrahedron Lett.*, 1991, **32**, 233.

97. A.E. DeCamp, A.T. Kawaguchi, R.P. Volante and I. Shinkai, *Tetrahedron Lett.*, 1991, **32**, 1867.

98. A.H. Fray, R.L. Kaye and E.F. Kleinman, *J. Org. Chem.*, 1986, **51**, 4828.

99. T. Nishi, M. Kataoka and Y. Morisawa, *Chem. Lett.*, 1989, 1993.

100. R.V. Hoffman and H-O. Kim, *Tetrahedron Lett.*, 1992, **33**, 3579.

101. H-E. Radunz, V. Eiermann, G. Schneider and A. Riethmuller, *Tetrahedron*, 1991, **47**, 1887.

102. W.D. Lubell and H. Rapoport, *J. Am. Chem. Soc.*, 1988, **110**, 7447.

103. M. Sakurai, T. Hata and Y. Yabe, *Tetrahedron Lett.*, 1993, **34**, 5939.

104. B.R. Lagu and D.C. Liotta, *Tetrahedron Lett.*, 1994, **35**, 547; Abstracts of papers of the *Amer. Chem. Soc.*, 1994, **207**, 140.

105. T. Yokomatsu, Y. Yuasa and S. Shibuya, *Heterocycles*, 1992, **33**, 1051.

106. H. Kotsuki, A. Miyazaki and M. Ochi, *Tetrahedron Lett.*, 1991, **32**, 4503.

107. D. Melon, C. Gravierpelletier, Y. le Merrer and J.C. Depezay, *Bull. Soc. Chim. Fr*, 1992, **129**, 585.

108. M. Sakurai, F. Saito, Y. Ohata, Y. Yabe and T. Nishi, *J. Chem. Soc., Chem. Commun.*, 1992, 1562.

109. G.B. Dreyer, D.M. Lambert, T.D. Meek, T.J. Carr, T.A. Tomaszek, A.V. Fernandez, H. Bartus, E. Cacciavillani, A.M. Hasell, M. Minnich, S.R. Petteway and B.W. Metcalf, *Biochemistry*, 1992, **31**, 6646.

110. M.D. Varney, K. Appelt, V. Kalish, M.R. Reddy, J. Tatlock, C.L. Palmer, W.H. Romines, B-W. Wu and L. Musick, *J. Med. Chem.*, 1994, **37**, 2274.

111. W.J. Thompson, R.G. Ball, P.L. Darke, J.A. Zugay and J.E. Thies, *Tetrahedron Lett.*, 1992, **33**, 2957.

112. M.T. Konieczny, P.H. Toma and M. Cushman, *J. Org. Chem.*, 1993, **58**, 4619.

113. M. Sakurai, S. Higashida, M. Sugano, T. Nishi, F. Saito, Y. Ohata, H. Handa, T. Komai, R. Yagi, T. Nishigaki and Y. Yabe, *Chem. Pharm. Bull.*, 1993, **41**, 1378.

114. W.J. Thompson, P.M.D. Fitzgerald, M.K. Holloway, E.A. Emini, P.L. Darke, B.M. McKeever, W.A. Schleif, J.C. Quintero, J.A. Zugay, T.J. Tucker, J.E. Schwering, C.F. Homnick, J. Numberg, J.P. Springer and J.R. Huff, *J. Med. Chem.*, 1992, **35**, 1685.

115. D. Askin, M.A. Wallace, J.P. Vacca, R.A. Reamer, R.P. Volante and I. Shinkai, *J. Org. Chem.*, 1992, **57**, 2771.

116. S.K. Thompson, A.M. Eppley, J.S. Frazee, M.G. Darcy, R.T. Lum, T.A. Tomaszek, L.A. Ivanoff, J.F. Morris, E.J. Sternberg, D.M. Lambert, A.V. Fernandez, S.R. Petteway, T.D. Meek, B.W. Metcalf and J.G. Gleason, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2441.

117. S.K. Thompson, K.H.M. Murthy, B. Zhao, E. Winborne, D.W. Green, S.M. Fisher, R.L. Des Jarlais, T.A. Tomaszek, T.D. Meek. J.G. Gleason, and S.S. Abdel-Meguid, *J. Med. Chem.*, 1994, **37**, 3100.

118. C.K. Chu, J.W. Beach, L.S. Jeong, B.G. Choi, F.I. Comer, A.J. Alves and, R.F. Schinazi, *J. Org. Chem.*, 1991, **56**, 6503.

119. T.K. Chakraborty and K.K. Gangakhedkar, *Tetrahedron Lett.*, 1991, **32**, 1897.

120. P.A. Grieco, T. Oguri and Y. Yokoyama, *Tetrahedron Lett.*, 1978, **25**, 419.

121. J.A. Martin, *Antiviral Res.*, 1992, **17**, 265.

122. Review, J.R. Huff, *J. Med. Chem.*, 1991, **34,** 2305.

123. C.L. Waller, T.I. Oprea, A. Giolitti and G.R. Marshall, *J. Med. Chem.*, 1993, **36,** 4152.

124. a) S. Natarajan, E.M. Gordon, E.F. Sabo, J.D. Godfrey, H.N. Weller, J. Pluscec, M. B. Rom and D.W. Cushman, *Biochem. Biophys. Res. Commun.*, 1984, **124,** 141.

b) E.M. Gorden, S. Natarajan, J. Pluscec, H.N. Weller, J.D. Godfrey, Jr., M.B. Rom, E.F. Sabo, J. Engebrecht, and D.W. Cushman, *Biochem. Biophys. Res. Commun.*, 1984, **124**, 148.

c) E.M. Gordon, J.D. Godfrey, Jr., J. Pluscec, D. von Langen and S. Natarajan, *Biochem. Biophys. Res. Commun.*, 1985, **126**, 419.

d) J.D. Godfrey, Jr., E.M. Gordon, D. von Langen, J. Engebrecht and J. Pluscec, *J. Org. Chem.*, 1986, **51**, 3075.

e) J.D. Godfrey, Jr., E.M. Gordon, D.J. von Langen, *Tetrahedron Lett.*, 1987, **28,** 1603.

125. J.G. Dann, D.K. Stammers, C.J. Harris, R.J. Arrowsmith, D.E. Davies, G.W. Hardy and J.A. Morton, *Biochem. Biophys. Res. Commun.*, 1986, **134,** 71; R.J. Arrowsmith, D.E. Davies, Y.C. Fogden, C.J. Harris and C. Thompson, *Tetrahedron Lett.*, 1987, **28**, 5569.

126. A.L. Swai, M.M. Miller, J. Green, D.H. Rich, J. Schneider, S.B.H. Kent and A. Wlodawer, *Proc. Natl. Acad. Sci. USA.*, 1990, **87,** 8805; M. Bacaand B.H. Kent, *Proc. Natl. Acad. Sci. USA.*, 1993, **90,** 11638.

127. P.F. Alewood, R.I. Brinkworth, R.J. Dancer, B. Garnharn, A.Jones and S.B.H. Kent, *Tetrahedron Lett.*, 1992, **33,** 977.

128. P. Cieplak and P.A. Kollman, *Computer-Aided Molecular Design*, 1993, **7,** 291.

129. N.A. Roberts, J.A. Martin, D. Kinchington, A.V. Broadhurst, J.C. Craig, I.B. Duncan, S.A. Galpin, B.K. Handa, J. Kay, A. Kröhn, R.W. Lambert, J.H. Merrett, J.S. Mills, K.E.B. Parkes, S. Redshaw, A.J. Ritchie, D.L. Taylor, G.J.

Thomas and P.J. Machin, *Science*, 1990, **248,** 358; A. Kröhn, S. Redshaw and J.C. Ritchie, *J. Med. Chem.*, 1991, **34,** 3342.

130. D.H. Rich, J.Green, M.V. Toth, G.R. Marshall and S.B.H. Kent, *J. Med. Chem.*, 1990, **33**, 1285.

131. a) D.H. Rich, C-Q. Sun, J.V.N.V. Prasad, A. Pathiasseril, M.V. Toth, G.R. Marshall, M. Clare, R.A. Mueller and K. Houseman, *J. Med. Chem.*, 1991, **34,** 1222.

b) D.H. Rich, J.V.N.V. Prasad, C-Q. Sun, J. Green, R. Mueller, K. Houseman, D. Macknezie and M. Malkovsky, *J. Med. Chem.*, 1992, **35**, 3803.

132. T.J. Tucker, W.C. Lumma, L.S. Payne, J.M. Wai, S.J. de Solms, E.A. Giuliani, P.L. Darke, J.C. Heimbach, J.A. Zugay, W.A. Schleif, J-C. Quintero, E.A. Enuni, J.R. Huff and P.S. Anderson, *J. Med. Chem.*, 1992, **35**, 2525.

133. H.L. Sham, D.A. Betebenner, C. Zhoa, N.E. Wideburg, A. Saldivar, D.J. Kempf, J.J. Plattner and D.W. Norbeck, *J. Chem. Soc., Chem. Commun.*, 1993, 1052.

134. J.C. Barrish, E.M. Gordon *et al, J. Med. Chem.*, 1994, **37,** 1758.

135. B.M. Kim, J.R. Huff *et al, Bioorg. Med. Chem. Lett.*, 1994, **4,** 2273.

136. K.E.B. Parkes, D.J. Bushnell, P.H. Crackett, S.J. Dunsdon, A.C. Freeman, M.P. Gunn, R.A. Hopkins, R.W. Lambert, J.A. Martin, J.H. Merrett, S. Redshaw, W.C. Spurden and G.J. Thomas, *J. Org. Chem.*, 1994, **59**, 3656.

137. D. Tourwe, J. Piron, P. Defreyn and G. van Binst, *Tetrahedron Lett.*, 1993, **34,** 5499.

138. B.M. Kim, J.R. Huff *et al, Bioorg. Med. Chem. Lett.*, 1994, **4,** 2199.

139. R. Herranz, M.L. Suarez-Gea, S. Vinuesa, M.T. Garcia-López and A. Martinez, *Tetrahedron Lett.*, 1991, **32**, 7579.

140. D.G. Hangauer, Merck Sharp and Dohme Research Laboratories, West Point, PA, unpublished results.

141. H. Maehr, *J. Chem. Educ.*, 1985, **62**, 114.

142. J. Jurczak and A. Golebiowski, *Chem. Rev.*, 1989, **89**, 149.

143. a) C.F. Stanfield, J.E. Parker and P. Kanellis, *J. Org. Chem.*, 1981, **46**, 4799.

b) K. Ramasamay, R.K. Olsen and T. Emery, *J. Org. Chem.*, 1981, **46**, 5438.

c) J-A. Fehrentz and B. Castro, *Synthesis*, 1983, 676.

d) C.F. Stanfield, J.E. Parker and P. Kanellis, *J. Org. Chem.*, 1981, **46**, 4797.

e) Y. Hamada and T. Shiori, *Chem. Pharm. Bull.*, 1982, **30**, 1921.

f.) K.E. Rittle, C.F. Homnick, G.S. Ponticello and B.E. Evans, *J. Org. Chem.*, 1982, **47**, 3016.

g) A. Golebiowski, U. Jacobsson and J. Jurczak, *Tetrahedron*, 1987, **43**, 3063.

h) J.R. Luly, J.F. Dellaris, J.J. Plattner, J.L. Soderquist and N.Yi, *J. Org. Chem.*, 1987, **52**, 1487.

144. a) D.H. Rich, B.J. Moon and A.S. Boparai, *J. Org. Chem.*, 1980, **45**, 2288.

b) A. Ito, R. Takahashi and Y. Baba, *Chem. Pharm. Bull.*, 1975, **23**, 3081.

c) R. Nishizawa and T. Saino, *J. Med. Chem.*, 1977, **20**, 510.

145. a) Y. Becker, A. Eisenstadt and J.K. Stille, *J. Org. Chem.*, 1980, **45**, 2145.

b) Y. Amino and K. Izawa, *Bull. Chem. Soc. Jpn.*, 1991, **64**, 613.

146. a) C. Meier and G. Boche, *Chem. Ber.*, 1990, **123**, 1691;

b) M. Iwata and H. Kuzuhara, *Chem . Lett.*, 1989, 2029;

c) P.G. Williard and S.E. de Laszlo, *J. Org. Chem.*, 1984, **49**, 3489;

d) C. Germon, A. Alexakis and J.F. Normant, *Synthesis*, 1984, 40.

147. a) S.R. Lammert and S. Kukolja, *J. Am. Chem. Soc.*, 1975, **97**, 5582;

b) A.I. Meyers, J.P. Lawson and D.R. Carver, *J. Org. Chem.*, 1981, **46**, 3123.

148. J.O. Osby, M.G. Martin and B. Ganem, *Tetrahedron Lett.*, 1984, **25**, 2093.

149. N.M. Yoon, C.S. Pak, H.C. Brown, S. Krishnamurthy and T.P. Stocky, *J. Org. Chem.*, 1973, **38**, 2786.

150. H.C. Brown, S. Krishnamurthy and R.A. Coleman, *J. Am. Chem. Soc.*, 1972, **94**, 1750.

151. T. Sasaki, K. Minamoto and H. Itoh, *J. Org. Chem.*, 1978, **43**, 2320.

152. A.K. Bose, F. Greer and C.C. Price, *J. Org. Chem.*, 1958, **23**, 1335.

153. H.C. Brown, *Synthesis*, 1979, 705.

154. E.J. Corey and G. Schmidt, *Tetrahedron Lett.*, 1979, **20**, 399.

155. E.J. Corey and J.W. Suggs, *Tetrahedron Lett.*, 1975, **16**, 2647.

156. S.G. Pyne, M.J. Hensel and P.L. Fuchs, *J. Am. Chem. Soc.*, 1982, **104**, 5719.

157. Y-S. Cheng, W-L. Liu and S-H. Chen, *Synthesis*, 1980,223.

158. R.D. Little, G.W. Muller, M.G. Venegas, G.L. Carroll, A. Bukhari, L. Patton and K. Stone, *Tetrahedron*, 1981, **37**, 4371; M.J. Kurth, M.J. O'Brien, H. Hope and M. Yanuck, *J. Org. Chem.*, 1985, **50**, 2626.

159. T.T. Tidwell, *Synthesis*, 1990, 857; A.J. Mancuso and D. Swern, *Synthesis*, 1981, 165.

160 J.R. Parikh and W.V.E. Doering, *J. Am. Chem. Soc.*, 1967, **89**, 2416; *J. Am. Chem. Soc.*, 1967, **89**, 5505.

161. A.J. Mancuso, S-L. Huang and D. Swern, *J. Org. Chem.*, 1978, **43**, 2480.

162. D.M. Walba, W.N. Thurmes and R.C. Haltiwanger, *J. Org. Chem.*, 1988, **53**, 1046.

163. W.P. Griffith, S.V. Ley, A.P. Whitcombe and A.D. White, *Chem. Commun.*, 1987, 1625; *Aldrichim. Acta.*, 1988, **21**, 16.

164. M.T. Reetz and J. Binder, *Tetrahedron Lett.*, 1989, **30**, 5425.

165. M.T. Reetz, M.W. Drewes and A. Schmitz, *Angew. Chem. Int. Ed. Engl.*, 1987, **26**, 1142.

166. L. Velluz, G. Amiard and R. Heymès, *Bull. Soc. Chim. Fr.*, 1954, 1012.

167. M.T. Reetz and M.W. Drewes, *Chem. Abs.*; 98014m:112.

168. D.H. Rich, E.T. Sun and A.S. Boparai, *J. Org. Chem.*, 1978, **43**, 3624.

169. M.T. Reetz and M.W. Drewes, *Chem. Abs.*, 111060-49-2 ; 37185p:108

170. Q. Liu, M.J. Simms, N. Boden and C.M. Rayner, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1363.

**Olefination**

171. D.J. Peterson, *J. Org. Chem.*, 1968, **33**, 780.

172. B.M.Trost, *Acc. Chem. Res.*, 1974, **7**, 85.

173. M. Julia and J-M. Paris, *Tetrahedron Lett.*, 1973, **14**, 4833.

174. A. Pelter, B. Singaram and J.W. Wilson, *Tetrahedron Lett.*, 1983, **24**, 635.

175. T. Okazoe, K. Takai and K. Utimoto, *J. Am. Chem. Soc.*, 1987, **109**, 951.

176. K. Takai, Y. Hotta, K. Oshima and H. Nozaki, *Tetrahedron Lett.*, 1978, **19**, 2417.

177. N.A. Petasis and E.I. Bzowey, *J. Am. Chem. Soc.*, 1990, **112**, 6392.

178. J. Baruenga, J.L. Fernández-Simón, J.M. Concellón and M. Yus, *J. Chem. Soc., Chem. Commun.*, 1986, 1665.

179. E. Vedejs, J.M. Doldhin and W.T. Stole, *J. Am. Chem. Soc.*, 1979, **101**, 249.

180. R.L. Sowerby and R.M. Coates, *J. Am. Chem. Soc.*, 1972, **94**, 4758.

181. Reviews; a)B.E. Maryanoff and A.B. Reitz, *Chem. Rev.*, 1989, **89**, 863-927 and references citied therein;

b) H.J. Bestmann, *Pure Appl. Chem.*, 1979, **51**, 515-533.

182. R. Greenwald, M. Chaykovsky and E.J. Corey, *J. Org. Chem.*, 1963, **28**, 1128.

183. T.W. Kwon, P.F. Keusenkothen and M.B. Smith, *J. Org. Chem.*, 1992, **57**, 6169.

184. H.J. Bestmann, W. Stransky and O. Vostrwosky, *Chem. Ber.*, 1976, **109**, 1694; H. Niwa, H. Inagki and K. Yamada, *Tetrahedron Lett.*, 1991, **32**, 5127.

185. W.S. Wadsworth and W.D. Emmons, *J. Am. Chem. Soc.*, 1961, **83**, 1733.

186. D.H. Wadsworth, O.E. Schupp, E.J. Seus and J.A. Ford, *J. Org. Chem.*, 1965, **30**, 680;

187. P.J. Murphy and J. Brennan, *Chem. Soc. Rev.*, 1988, **17**, 1.

188. H.J. Bestmann, *Angew. Chem., Int. Ed. Engl.*, 1965, **4**, 583.

189. U. Wannaqat and H. Niederprüm, *Chem. Ber.*, 1961, **94**, 1540.

190. G. Wittig, H. Eggersand and P. Duffner, *Liebigs Ann. Chem.*, 1958, **10**, 619.

191. A.H. Hoveyda, D.A. Evans and G.C. Fu, *Chem. Rev.*, 1993, **93**, 1307-70.

192. A. Albeck and R. Persky, and references therein *J. Org. Chem.*, 1994, **59**, 653.

193. H. Kogen and T. Nishi, and references therein, *J. Chem. Soc., Chem. Commun.*, 1987, 311.

194. K. Hori and Y. Ohfune, *J. Org. Chem.*, 1988, **53**, 3886.

195. A. Jenmalm, W. Berts, Y-L. Li, K. Luthman, I. Csöregh and U. Hacksell, *J. Org. Chem.*, 1994, **59**, 1139.

196. S. Romeo and D.H. Rich, *Tetrahedron Lett.*, 1994, **35**, 4939.

197. E.J. Corey, M. Jautelat and W. Oppolzer, *Tetrahedron Lett.*, 1967, **24**, 2325.

198. E. Borredon, M. Delmas and A. Gaset, *Tetrahedron Lett.*, 1982, **23**, 5283.

199. A. Merz and G. Markl, *Angew. Chem. Int. Ed.Engl.*, 1973, **12**, 845.

200. C.D. Gutshe, *Org. React.* 1954, **8**, 364-429.

201. J.R. Luly, N. Yi, J. Soderquist, H. Stein, J. Cohen, T.J. Perun and J.J. Plattner, *J. Med. Chem.*, 1987, **30**, 1609; J.R. Luly, J.J. Plattner, H. Stein, N. Yi, J. Soderquist, P.A. Marcotte, H.D. Kleinert and T.J. Perun, *Biochem. Biophys. Res. Comm.*, 1987, **143**, 44.

202. D.J. Kempf, E. de Lara, H. Stein, J. Cohen and J.J. Plattner, *J. Med. Chem.*, 1987, **30**, 1978.

203. S.H. Rosenberg, J.J. Plattner, K.W. Woods, H.H. Stein, P.A. Marcotte, J. Cohen and T.J. Perun, *J. Med. Chem.*, 1987, **30**, 1228.

204. J-I. Yamada, M. Yumoto and Y. Yamamoto, *Tetrahedron Lett.*, 1989, **30**, 4255 and references sitied therein.

205. I.E. Overman, L.A. Flippin, *Tetrahedron Lett.*, 1981, **22**, 195.

206. Y. Yamamoto, N. Asao, M. Meguro, N. Tsukada, H. Nemoto, N. Sadayori, J.G. Wilson and H. Nakamura, *J. Chem. Soc., Chem. Commun.*, 1993, 1201.

207. M. Meguro, N. Asao and Y. Yamamoto, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2597

208. K.I. Sutowardoyo, M. Emziane, P. L'hoste and D. Sinou, *Tetrahedron*, 1991, **47**, 1435.

209. G.H. Posner and D.Z. Rogers, *J. Am. Chem. Soc.*, 1977, **99**, 195.

210 M. Poch, M. Alcón, A. Moyano, M.A. Pericàs and A. Riera, *Tetrahedron Lett.*, 1993, **34**, 7781; M. Canas, M. Poch, X. Verdaguer, A. Moyano, M.A. Pericàs and A. Riera, *Tetrahedron Lett.*, 1991, **32**, 6931.

211. M. Caron and K.B. Sharpless, *J. Org. Chem.*, 1985, **50**, 1560; J.M. Chong and K.B. Sharpless, *J. Org. Chem.*, 1985, **50**, 1563.

212. J. Moulines, P. Charpentier, J-P. Bats, A. Nuhrich and A-M. Lamidey, *Tetrahedron Lett.*, 1992, **33**, 487.

213. J. Kagan, B.E. Firth, N.Y. Shih and C.G. Boyajian, *J. Org. Chem.*, 1977, **42**, 343.

214. C-L. Spawn, G.L. Dritina and D.F. Wiemer, *Synthesis*, 1986, 315.

215. N. Takaishi, H. Takaishi and Y. Inamoto, *Tetrahedron Lett.*, 1985, **26**, 2361.

216. M. Fiorenza, A. Ricci, M. Taddei and D. Tassi, *Synthesis*, 1983, 640.

217. K.I. Sutowardoyo, M. Emziane, P. Lhoste and D. Sinou, *Tetrahedron Lett.*, 1991, **32**, 1435; S. Matsubara, H. Onishi and K. Utimoto, *Tetrahedron Lett.*, 1990, **31**, 6209; K. Imi, N. Yanagihara and K. Utimoto, *J. Org. Chem.*, 1987, **52**, 1013; G.O. Spessard, A.R. Ritter, D.M. Johnson and A.M. Montgomery, *Tetrahedron Lett.*, 1983, **24**, 655 and M.F. Semmelhack, J.J. Bozell, W. Wulff, E. Spiess and A. Zask, *J. Am. Chem. Soc.*, 1982, **104**, 5850.

218. Y. Fujimoto, Y. Kanzawa, Y. Ikuina, K. Kakinuma and N. Ikekawa, *J. Chem. Soc., Chem. Commun.*, 1989, 1107.

219. J.A. Ciaccio, K.J. Addess and T.W. Bell, *Tetrahedron Lett.*, 1986, **27**, 3697.

220. M. Shimizu, A. Yoshida and T. Fujisawa, *Synlett.*, 1992, 204.

221. J. Gorzynshi-Smith, *Synthesis*, 1984, 629.

222. G-S. Lin, PhD, Thesis, Bath University, 1993.

223. K. Filbey, Peptide mimetics, Final Year Project, Bath University, 1992.

224. Y-G. Suh, B-A. Koo, J-A. Ko and Y-S. Cho, *Chem. Lett.*, 1993, 1907.

225. J.S. Bajwa and R.C. Anderson, *Tetrahedron Lett.*, 1991, **26**, 3021.

226. J. Barluenga, B. Baragana, A. Alonso and J.M. Concellón, 1994, **8**, 969.

227. E.J. Corey, D.A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Sanvelsson and S. Hammarstom, *J. Am. Chem. Soc.*, 1980, **102**, 3663.

228. J.F. Dellaria, R.G. Maki, B.A. Bopp, J. Cohen, H.D. Kleinert, J.R. Luly, I. Merits, J.J. Plattner and H.H. Stein, *J. Med. Chem.*, 1987, **30**, 2137.

229. J.R. Luly, G.Bolis, N. BaMaung, J. Soderquist, J.F. Dellaria, H.H. Stein, J. Cohen , T.J. Perun, J. Greer and J.J. Plattner, *J. Med. Chem.*, 1988, **31**, 532.

230. D.A. Schooley, M. Koreeda and J. Dillon, *J. Chem. Soc., Chem. Commun.*, 1971, 1235.

231. G.I. Poos, G.E. Arth, R.E. Beyler and I.H. Sarett, *J. Am. Chem. Soc.*, 1953, **75**, 422.

232. S. Danishefsky, J. Aubé and M. Bednarski, *J. Am. Chem. Soc.*, 1986, **108**, 4145.

233. R. Baker, P.D. Leeson, N.J. Liverton and J.J. Kulagowski, *J. Chem. Soc., Chem. Commun.*, 1990, 462.

234. T. Katagiri, F. Obara, S. Toda and K. Furuhashi, *Synlett.*, 1994, 507.

235. McBee, C.E. Hathaway and C.W. Roberts, *J. Am. Chem. Soc.*, 1956, **78**, 3851.

236. Y-J. Liu, T-Y. Chu and R. Engel, *Synth. Commun.*, 1992, **22**, 2367.

237. G. Lauterbach, G. Posselt, R. Schäfer and D. Schnurpfeil, *J. Fur. Prakt. Chemie*, 1981, 101.

238. Y. Pocker, B.P. Ronald and K.W. Anderson, *J. Am. Chem. Soc.*, 1988, **110**, 6492.

239. F.A. Long and J.G. Pritchard, *J. Am. Chem. Soc.*, 1956, **78**, 2663.

240. W.R. Roush, J.A. Straub and R.J. Brown, *J. Org. Chem.*, 1987, **52**, 5127.

241. a) M. Wills, Bath University, discussion.

b) J.E. Baldwin, *J. Chem. Soc., Chem. Comm.*, 1976, 734.

242. T. Ogino, *Tetrahedron Lett.*, 1980, **21**, 177; T. Ogino and K. Mochizuki, *Chem. Lett.*, 1979, 443.

243. V. Bhushan, R. Rathore and S. Chandrasekaran, *Synthesis*, 1984, 431.

244. R. Ray and D.S. Matterson, *Tetrahedron Lett.*, 1980, **21**, 449.

245. R. Hanselaer, M.Samson and M. van de Walle, *Tetrahedron*, 1978, **34**, 2393.

246. V. VanRheenen, R.C. Kelly and D.Y. Cha, *Tetrahedron Lett.*, 1976, **23**, 1973.

247. P.J. Stang, M. Hanack and L.R. Subramanian, *Synthesis*, 1982, 85.

248. J. Lerouxs and A.S. Perkin, *Carbohydr. Res.*, 1976, **47**, C8.

249. E. Vedejs, D.A. Engler and M.J. Mullins, *J. Org. Chem.*, 1977, **42**, 3109.

250. F. Effenberger, U. Burkard and J. Willfahrt, *Angew Chem., Int. Ed. Engl.*, 1983, **22**, 65.

251. R.W. Feenstra, E.H.M. Stokkingreef, R.J.F. Nivard and H.C.J. Ottenheijm, *Tetrahedron*, 1988, **44**, 5583.

252. T.P. Kogan, T.C. Somers and M.C. Venuti, *Tetrahedron*, 1990, **46**, 6623.

253. F. Degerbeck, B. Fransson, L. Grehn and U. Ragnarsson, *J. Chem. Soc., Perkin. Trans. 1*, 1992, 245; *ibid*, 1993, 11.

254. G.H. Hakimelahi and G. Just, *Tetrahedron Lett.*, 1979, **20**, 3643.

255. J.F. Dellaria and R.G. Maki, *Tetrahedron Lett.*, 1986, **27**, 2337.

256. P.K. Chakravarty, P. Combs, A. Roth and W.J. Greenlee, *Tetrahedron Lett.*, 1987, **28**, 611.

257. S. Patai, *The Chemistry of the Carbon-Nitrogen Double Bond*, Interscience Publishers, 1970, p. 64.

258. H. Weingarten, J.P. Chupp and W.A. White, *J. Org. Chem.*, 1967, **32**, 3246.

259. R. Bonnett and T.R. Emerson, *J. Chem. Soc.*, 1965, 4508.

260. B.T. Cho and Y.S. Chun, *J. Chem. Soc., Perkin. Trans. 1*, 1990, 3200.

261. R.F. Borch, M.D. Bernstein and H.D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897.

262. A.F. Abdel-Magid, C.A. Maryanoff and K.G. Carson, *Tetrahedron Lett.*, 1990, **31**, 5595.

263. Y. Watanabe, M. Yamashita, T-A. Mitsudo, M. Tanaka and Y. Takegami, *Tetrahedron Lett.*, 1974, **22**, 1879.

264. S. Murata, M. Suzuki and R. Noyori, *J. Am. Chem. Soc.*, 1979, **101**, 2739

265. D.W. Brown, A.J. Floyd and M. Sainsbury, *Organic Spectroscopy*, Wiley, Bath, 1988, Chpt 4.16.

266. G.W Kabalka, M. Varma and R.S. Varma, *J. Org. Chem.*, 1986, **51**, 2388.

267. R.K. Crossland and K.L. Servis, *J. Org. Chem.*, 1970, **35**, 3195.

268. O. Mitsunobu, *Synthesis*, 1981, 1-28.

269. D.L. Hughes, *Org. React.*, Vol. 42, ed. L.A. Paquette *et al*, 1992, Wiley.

270. M.L. Edwards, D.M. Stemerick and J.R.McCarthy, *Tetrahedron Lett.*, 1990, **31**, 3417.

271. P.G. Sammes and S. Smith, *J. Chem. Soc., Perkin. Trans. 1*, 1984, 2145.

272. T. Tsunoda, Y. Yamamiya and S. Itô, *Tetrahedron Lett.*, 1993, **34**, 1639.

273. H. Newman, *J. Org. Chem.*, 1965, **30**, 1287.

274. Glaxo Group Research, Ware.

275. S. Hanessian and P. Lavallee, *Can. J. Chem.*, 1975, **53**, 2975.

276. S. Hanessian and P. Lavallee, *Can. J. Chem.*, 1977, **55**, 562.

277. E.J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, 1972, **94**, 6190.

278. M.J. Genin and R.L. Johnson, *J. Am. Chem. Soc.*, 1992, **114**, 8878.

279. Biosym. Technologies Inc. (San Diego, CA)

280. M.J. Frisch, G.W. Trucks, M. Head-Gordon, P.M.W. Gill, M.W. Wong, J.B. Foresman, B.G. Johnson, H.B. Schlegel, M.A. Robb, E.S. Replogle, R. Gomperts, J.L. Andres, K. Raghavachari, J.S. Binkley, C. Gonzalez, R.L. Martin, D.J. Fox, D.J. Defrees, J. Baker, J.J.P. Stewart and J.A. Pople, Gaussian 92, Revision C.4, Gaussian, Inc., Pittsburgh PA, 1992.

281. P. Dauber-Osguthorpe, D.K. Jones, M.M. Campbell and D.J. Osguthorpe, *Int. J. Pep. Protein Res.*, 1991, **38**, 357.

282. J.A. Allan, R.M. Hembry, S. Angal, J.J. Reynolds and G. Murphy, *J. Cell Sci.*, 1991, **99**, 789.

283. G. Murphy et al, *J. Biol. Chem.*, 1992, **267**, 9612.

284. L.M. Matrisian, *BioEssays*, 1992, **14**, 455; A.J.P. Docherty, J. O'Connell, T. Crabbe, S. Angal and G. Murphy, *Trends in Biotechnology*, 1992, **10**, 200.

285. B.W. Mathews *et al*, *Nature New Biol.*, 1972, **238**, 37; B.W. Mathews, *Acc. Chem. Res.*, 1988, 21, 333.

286. B. Lovejoy, A. Cleasby, A.M. Hassell, K. Longley, M.A. Luther, D. Weigl, G. McGeehan, A.B. McElroy, D. Drewry, M.H. Lambert and S.R. Jordan, *Science*, 1994, **263**, 375.

287. B. Lovejoy, A.M. Hassell, M.A. Luther, D. Weigl and S.R. Jordan, *Biochem.*, 1994, **33**, 8207.

288. P. Dauber-Osguthorpe, V.A. Roberts, D.J. Osguthorpe, J. Wolff, M. Genest and A.T. Hagler, *Proteins: Structure, Fuction and Genetics*, 1988, **4**, 31.

289 A.Vogel, *Textbook of Practical Organic Chemistry*, Longman Group Limited, London, 1978, 3rd Edn, p. 269.

290 D.D. Perrin, W.L.F. Armarego and D.R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1988, 3rd Edn.

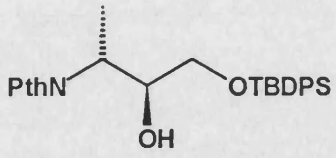
291 T.J. de Boer and H.J. Backer, *Org. Syntheses Coll.*, 1963, **Vol. 4.**, p. 250.

292. Sheldrick G.M., SHELX86, a computer program for crystal structure determination, University of Göttingen, 1986.

293. Sheldrick G.M., SHELX76, a computer program for crystal structure determination, University of Cambridge, 1976.

# APPENDIX ONE

## X-ray Crystallographic Data for (474)




(474)

**Figure 101**

*Crystal data*: $C_{28}H_{31}NO_4Si$, $M$ = 947.3, monoclinic, $a$ = 32.219(9), $b$ = 8.887(1), $c$ = 18.816(5)Å, $\beta$ = 110.66(2)°, $U$ = 5041.1 Å$^3$, space group $C2/c$, Z = 8, $D_c$ = 1.25gcm$^{-3}$, $\mu$(Mo-$K\alpha$) = 1.20cm$^{-1}$, $F$(000) = 2016. Data were measured at room temperature on a CAD4 automatic four-circle diffractometer in the range $2 \leq 3 \leq 24°$. 3409 reflections were collected of which 1460 were unique with $I \geq 2\sigma(I)$. Data were corrected for Lorentz and polarization but not for absorption. The structure was solved by Direct methods and refined using the SHELX[292,293] suite of programs. In the final least squares cycles all atoms were allowed to vibrate anisotropically. Hydrogen atoms were included at calculated positions except in the instance of the H3 (attached to O3). This proton was located in an advanced Difference Fourier and refined at a distance of 0.92 Å from the parent atom.

Final residuals after 12 cycles of least squares were $R$ = 0.0512, $Rw$ = 0.0453, for a weighting scheme of $w = 2.6106/[\sigma^2(F) + 0.000482(F)^2]$. Max. final shift/esd was 0.018. The max. and min. residual densities were 0.10 and -0.08 eÅ$^{-3}$ respectively. Final fractional atomic coordinates and isotropic thermal parameters, bond distances and angles are given in **Tables 33**, **34** and **35** respectively. Tables of anisotropic temperature factors are available as supplementary data. The asymmetric unit is shown in **Figure 101**, along with the labelling scheme used.

## Table 31

Fractional atomic co-ordinates (x104) for (474)

| | x | y | z |
|---|---|---|---|
| Si(1) | 3679(1) | 2224(2) | 3554(1) |
| O(1) | 4306(2) | 2492(5) | 6309(3) |
| O(2) | 3029(2) | -216(5) | 5347(3) |
| O(3) | 2907(2) | 4807(5) | 5080(2) |
| O(4) | 3560(1) | 2359(4) | 4336(2) |
| N(1) | 3608(2) | 1473(6) | 5831(3) |
| C(1) | 4062(3) | 1402(8) | 6117(4) |
| C(2) | 4187(2) | -193(7) | 6118(3) |
| C(3) | 4592(2) | -893(9) | 6355(4) |
| C(4) | 4602(3) | -2440(9) | 6284(4) |
| C(5) | 4215(3) | -3261(8) | 5993(4) |
| C(6) | 3804(2) | -2566(7) | 5747(3) |
| C(7) | 3802(2) | -1028(7) | 5819(3) |
| C(8) | 3422(2) | 61(8) | 5625(4) |
| C(9) | 3355(2) | 2861(8) | 5800(3) |
| C(10) | 3121(2) | 3417(8) | 5048(4) |
| C(11) | 3320(2) | 3615(7) | 4459(4) |
| C(12) | 3146(2) | 2889(8) | 6390(3) |
| C(13) | 3974(2) | 394(6) | 3625(3) |
| C(14) | 4077(2) | 121(7) | 2893(3) |
| C(15) | 4413(2) | 400(7) | 4306(4) |
| C(16) | 3688(2) | -918(7) | 3715(4) |
| C(17) | 4059(2) | 3807(7) | 3528(4) |
| C(18) | 4284(2) | 4602(8) | 4188(4) |
| C(19) | 4585(3) | 5729(8) | 4210(5) |
| C(20) | 4672(3) | 6084(8) | 3570(6) |
| C(21) | 4459(3) | 5330(9) | 2917(5) |
| C(22) | 4157(2) | 4213(7) | 2894(4) |
| C(23) | 3148(2) | 2226(7) | 2731(3) |
| C(24) | 2780(2) | 1490(8) | 2799(4) |
| C(25) | 2379(2) | 1394(8) | 2197(5) |
| C(26) | 2330(2) | 2040(8) | 1513(4) |
| C(27) | 2684(2) | 2793(8) | 1435(4) |
| C(28) | 3083(2) | 2883(7) | 2031(3) |

**Table 32**

Anisotropic temperature factors (Å x103) for **(474)**

| | U11 | U22 | U33 | U23 | U13 | U12 |
|---|---|---|---|---|---|---|
| Si(1) | 47(1) | 48(1) | 42(1) | 1(1) | 24(1) | -1(1) |
| O(1) | 72(3) | 59(3) | 92(4) | -13(3) | 32(3) | -6(3) |
| O(2) | 57(3) | 70(3) | 87(4) | -1(3) | 21(3) | 4(3) |
| O(3) | 91(4) | 64(3) | 60(3) | -4(2) | 41(3) | 30(3) |
| O(4) | 58(3) | 45(2) | 49(2) | 2(2) | 31(2) | 12(2) |
| N(1) | 55(4) | 44(4) | 48(3) | 4(3) | 25(3) | 9(3) |
| C(1) | 52(5) | 58(5) | 46(4) | -4(4) | 22(4) | -2(4) |
| C(2) | 49(4) | 45(4) | 42(4) | 2(3) | 21(3) | 8(4) |
| C(3) | 60(5) | 69(6) | 75(5) | 9(4) | 32(4) | 8(4) |
| C(4) | 76(6) | 73(6) | 83(5) | 16(4) | 46(5) | 28(5) |
| C(5) | 89(6) | 54(5) | 71(5) | 9(4) | 40(5) | 20(5) |
| C(6) | 75(5) | 50(5) | 60(4) | 4(4) | 30(4) | 5(4) |
| C(7) | 52(5) | 43(4) | 42(4) | 6(3) | 20(4) | 16(4) |
| C(8) | 42(5) | 67(5) | 45(4) | 7(4) | 15(4) | -5(4) |
| C(9) | 93(5) | 59(5) | 57(4) | 13(4) | 40(4) | 33(4) |
| C(10) | 110(6) | 60(5) | 71(5) | 12(4) | 56(5) | 41(5) |
| C(11) | 84(6) | 62(5) | 70(5) | 4(4) | 44(4) | 20(4) |
| C(12) | 97(6) | 89(5) | 65(4) | 11(4) | 52(4) | 25(5) |
| C(13) | 46(4) | 51(4) | 40(4) | 2(3) | 26(4) | 3(3) |
| C(14) | 82(6) | 72(5) | 58(5) | -5(4) | 39(4) | 9(4) |
| C(15) | 70(5) | 74(5) | 62(5) | 5(4) | 29(4) | 16(4) |
| C(16) | 74(5) | 56(4) | 93(6) | 8(4) | 47(5) | 9(4) |
| C(17) | 44(4) | 45(4) | 55(4) | 5(4) | 24(4) | 3(3) |
| C(18) | 73(6) | 61(5) | 75(6) | -3(4) | 33(5) | -6(4) |
| C(19) | 80(6) | 59(5) | 108(7) | -19(5) | 34(5) | -24(5) |
| C(20) | 56(5) | 56(5) | 138(8) | 18(6) | 38(6) | -2(4) |
| C(21) | 62(6) | 86(6) | 92(6) | 33(5) | 32(5) | -2(5) |
| C(22) | 49(5) | 72(5) | 65(5) | 14(4) | 26(4) | -6(4) |
| C(23) | 52(4) | 48(4) | 47(4) | 2(3) | 32(3) | -1(3) |
| C(24) | 60(5) | 91(5) | 48(4) | 12(4) | 22(4) | -8(4) |
| C(25) | 57(5) | 95(6) | 72(5) | -3(5) | 25(5) | -19(4) |
| C(26) | 59(5) | 62(5) | 63(5) | -13(4) | 15(4) | 6(4) |
| C(27) | 72(5) | 79(5) | 53(4) | 13(4) | 23(4) | 0(5) |
| C(28) | 53(5) | 79(5) | 48(4) | 7(4) | 14(4) | -7(4) |

## Table 33

Hydrogen fractional atomic co-ordinates (x104) and isotropic temperature factors (Å2 x103) for **(474)**

| | x | y | z | U |
|---|---|---|---|---|
| H(3) | 3017(21) | 5231(69) | 5556(16) | 102(5) |
| H(31) | 4862(2) | -325(9) | 6564(4) | 102(5) |
| H(41) | 4883(3) | -2951(9) | 6440(4) | 102(5) |
| H(51) | 4231(3) | -4336(8) | 5959(4) | 102(5) |
| H(61) | 3534(2) | -3131(7) | 5537(3) | 102(5) |
| H(91) | 3556(2) | 3694(8) | 5963(3) | 102(5) |
| H(101) | 2942(2) | 2537(8) | 4869(4) | 102(5) |
| H(111) | 3519(2) | 4455(7) | 4603(4) | 102(5) |
| H(112) | 3084(2) | 3835(7) | 3989(4) | 102(5) |
| H(121) | 2984(2) | 3811(8) | 6351(3) | 102(5) |
| H(122) | 3372(2) | 2823(8) | 6885(3) | 102(5) |
| H(123) | 2947(2) | 2052(8) | 6316(3) | 102(5) |
| H(141) | 4257(2) | 930(7) | 2823(3) | 102(5) |
| H(142) | 3805(2) | 74(7) | 2465(3) | 102(5) |
| H(143) | 4235(2) | -812(7) | 2937(3) | 102(5) |
| H(151) | 4594(2) | 1220(7) | 4255(4) | 102(5) |
| H(152) | 4566(2) | -533(7) | 4320(4) | 102(5) |
| H(153) | 4352(2) | 517(7) | 4767(4) | 102(5) |
| H(161) | 3619(2) | -775(7) | 4166(4) | 102(5) |
| H(162) | 3848(2) | -1843(7) | 3753(4) | 102(5) |
| H(163) | 3419(2) | -958(7) | 3281(4) | 102(5) |
| H(181) | 4230(2) | 4361(8) | 4644(4) | 102(5) |
| H(191) | 4732(3) | 6262(8) | 4675(5) | 102(5) |
| H(201) | 4881(3) | 6860(8) | 3584(6) | 102(5) |
| H(211) | 4519(3) | 5574(9) | 2466(5) | 102(5) |
| H(221) | 4010(2) | 3701(7) | 2423(4) | 102(5) |
| H(241) | 2805(2) | 1039(8) | 3276(4) | 102(5) |
| H(251) | 2135(2) | 868(8) | 2261(5) | 102(5) |
| H(261) | 2053(2) | 1970(8) | 1095(4) | 102(5) |
| H(271) | 2653(2) | 3263(8) | 960(4) | 102(5) |
| H(281) | 3324(2) | 3419(7) | 1959(3) | 102(5) |

## Table 34

Bond lengths (Å) for **(474)**

| | | | |
|---|---|---|---|
| O(4)-Si(1) | 1.650(5) | C(13)-Si(1) | 1.864(8) |
| C(17)-Si(1) | 1.876(8) | C(23)-Si(1) | 1.860(8) |
| C(1)-O(1) | 1.220(8) | C(8)-O(2) | 1.214(8) |
| C(10)-O(3) | 1.427(8) | C(11)-O(4) | 1.423(7) |
| C(1)-N(1) | 1.368(8) | C(8)-N(1) | 1.386(8) |
| C(9)-N(1) | 1.469(8) | C(2)-C(1) | 1.473(10) |
| C(3)-C(2) | 1.371(9) | C(7)-C(2) | 1.382(9) |
| C(4)-C(3) | 1.383(10) | C(5)-C(4) | 1.381(10) |
| C(6)-C(5) | 1.384(9) | C(7)-C(6) | 1.374(9) |
| C(8)-C(7) | 1.501(10) | C(10)-C(9) | 1.435(9) |
| C(12)-C(9) | 1.488(8) | C(11)-C(10) | 1.471(9) |
| C(14)-C(13) | 1.548(9) | C(15)-C(13) | 1.537(10) |
| C(16)-C(13) | 1.531(9) | C(18)-C(17) | 1.390(9) |
| C(22)-C(17) | 1.385(9) | C(19)-C(18) | 1.382(10) |
| C(20)-C(19) | 1.366(11) | C(21)-C(20) | 1.355(10) |
| C(22)-C(21) | 1.380(9) | C(24)-C(23) | 1.399(8) |
| C(28)-C(23) | 1.388(8) | C(25)-C(24) | 1.386(9) |
| C(26)-C(25) | 1.366(9) | C(27)-C(26) | 1.374(9) |
| C(28)-C(27) | 1.377(9) | H(3)-O(3) | 0.920(2) |
| H(31)-C(3) | 0.960 | H(41)-C(4) | 0.960 |
| H(51)-C(5) | 0.960 | H(61)-C(6) | 0.960 |
| H(91)-C(9) | 0.960 | H(101)-C(10) | 0.960 |
| H(111)-C(11) | 0.960 | H(112)-C(11) | 0.960 |
| H(121)-C(12) | 0.960 | H(122)-C(12) | 0.960 |
| H(123)-C(12) | 0.960 | H(141)-C(14) | 0.960 |
| H(142)-C(14) | 0.960 | H(143)-C(14) | 0.960 |
| H(151)-C(15) | 0.960 | H(152)-C(15) | 0.960 |
| H(153)-C(15) | 0.960 | H(161)-C(16) | 0.960 |
| H(162)-C(16) | 0.960 | H(163)-C(16) | 0.960 |
| H(181)-C(18) | 0.960 | H(191)-C(19) | 0.960 |
| H(201)-C(20) | 0.960 | H(211)-C(21) | 0.960 |
| H(221)-C(22) | 0.960 | H(241)-C(24) | 0.960 |
| H(251)-C(25) | 0.960 | H(261)-C(26) | 0.960 |
| H(271)-C(27) | 0.960 | H(281)-C(28) | 0.960 |

## Table 35

Bond angles (deg.) for **(474)**

| | | | |
|---|---|---|---|
| C(13)-Si(1)-O(4) | 105.8(3) | C(17)-Si(1)-O(4) | 109.2(3) |
| C(17)-Si(1)-C(13) | 109.6(3) | C(23)-Si(1)-O(4) | 107.8(3) |
| C(23)-Si(1)-C(13) | 110.8(4) | C(23)-Si(1)-C(17) | 113.3(4) |
| C(11)-O(4)-Si(1) | 120.9(4) | C(8)-N(1)-C(1) | 111.3(7) |
| C(9)-N(1)-C(1) | 123.8(7) | C(9)-N(1)-C(8) | 124.8(6) |
| N(1)-C(1)-O(1) | 124.6(7) | C(2)-C(1)-O(1) | 128.0(7) |
| C(2)-C(1)-N(1) | 107.4(7) | C(3)-C(2)-C(1) | 131.8(7) |
| C(7)-C(2)-C(1) | 108.1(7) | C(7)-C(2)-C(3) | 120.2(7) |
| C(4)-C(3)-C(2) | 118.1(8) | C(5)-C(4)-C(3) | 121.0(8) |
| C(6)-C(5)-C(4) | 121.4(7) | C(7)-C(6)-C(5) | 116.6(7) |
| C(6)-C(7)-C(2) | 122.7(7) | C(8)-C(7)-C(2) | 106.8(7) |
| C(8)-C(7)-C(6) | 130.4(7) | N(1)-C(8)-O(2) | 125.9(7) |
| C(7)-C(8)-O(2) | 127.8(8) | C(7)-C(8)-N(1) | 106.3(6) |
| C(10)-C(9)-N(1) | 114.8(6) | C(12)-C(9)-N(1) | 112.0(6) |
| C(12)-C(9)-C(10) | 120.8(7) | C(9)-C(10)-O(3) | 110.4(6) |
| C(11)-C(10)-O(3) | 105.9(6) | C(11)-C(10)-C(9) | 124.6(7) |
| C(10)-C(11)-O(4) | 115.8(6) | C(14)-C(13)-Si(1) | 109.7(5) |
| C(15)-C(13)-Si(1) | 110.6(5) | C(15)-C(13)-C(14) | 108.5(6) |
| C(16)-C(13)-Si(1) | 111.2(5) | C(16)-C(13)-C(14) | 107.8(6) |
| C(16)-C(13)-C(15) | 108.9(6) | C(18)-C(17)-Si(1) | 119.9(6) |
| C(22)-C(17)-Si(1) | 124.5(6) | C(22)-C(17)-C(18) | 115.5(7) |
| C(19)-C(18)-C(17) | 122.3(8) | C(20)-C(19)-C(18) | 120.0(9) |
| C(21)-C(20)-C(19) | 119.3(9) | C(22)-C(21)-C(20) | 120.7(8) |
| C(21)-C(22)-C(17) | 122.2(8) | C(24)-C(23)-Si(1) | 119.0(6) |
| C(28)-C(23)-Si(1) | 125.3(5) | C(28)-C(23)-C(24) | 115.7(7) |
| C(25)-C(24)-C(23) | 121.9(7) | C(26)-C(25)-C(24) | 120.6(7) |
| C(27)-C(26)-C(25) | 118.6(7) | C(28)-C(27)-C(26) | 120.9(7) |
| C(27)-C(28)-C(23) | 122.2(7) | C(10)-O(3)-H(3) | 111.7(45) |
| H(31)-C(3)-C(2) | 120.9(5) | C(4)-C(3)-H(31) | 120.9(6) |
| H(41)-C(4)-C(3) | 119.5(6) | C(5)-C(4)-H(41) | 119.5(5) |
| H(51)-C(5)-C(4) | 119.3(5) | C(6)-C(5)-H(51) | 119.3(5) |
| H(61)-C(6)-C(5) | 121.7(5) | C(7)-C(6)-H(61) | 121.7(5) |
| H(91)-C(9)-N(1) | 109.3(5) | C(10)-C(9)-H(91) | 96.3(5) |
| C(12)-C(9)-H(91) | 100.4(5) | H(101)-C(10)-O(3) | 118.6(5) |
| H(101)-C(10)-C(9) | 96.1(5) | C(11)-C(10)-H(101) | 101.7(5) |
| H(111)-C(11)-O(4) | 107.9(4) | H(111)-C(11)-C(10) | 107.9(5) |
| H(112)-C(11)-O(4) | 107.9(4) | H(112)-C(11)-C(10) | 107.9(5) |
| H(112)-C(11)-H(111) | 109.5 | H(121)-C(12)-C(9) | 109.5(5) |
| H(122)-C(12)-C(9) | 109.5(5) | H(122)-C(12)-H(121) | 109.5 |
| H(123)-C(12)-C(9) | 109.5(5) | H(123)-C(12)-H(121) | 109.5 |
| H(123)-C(12)-H(122) | 109.5 | H(141)-C(14)-C(13) | 109.5(4) |
| H(142)-C(14)-C(13) | 109.5(4) | H(142)-C(14)-H(141) | 109.5 |

| | | | |
|---|---|---|---|
| H(101)-O(4) | 2.531 | H(111)-O(4) | 1.945 |
| H(112)-O(4) | 1.945 | C(13)-O(4) | 2.806 |
| C(17)-O(4) | 2.877 | H(181)-O(4) | 2.697 |
| C(23)-O(4) | 2.840 | C(2)-N(1) | 2.291 |
| C(7)-N(1) | 2.312 | H(91)-N(1) | 2.003 |
| C(10)-N(1) | 2.447 | H(101)-N(1) | 2.457 |
| C(11)-N(1) | 3.075 | C(12)-N(1) | 2.452 |
| H(122)-N(1) | 2.647 | H(123)-N(1) | 2.647 |
| C(3)-C(1) | 2.597 | C(7)-C(1) | 2.312 |
| C(8)-C(1) | 2.274 | C(9)-C(1) | 2.503 |
| H(91)-C(1) | 2.560 | H(31)-C(2) | 2.038 |
| C(4)-C(2) | 2.362 | C(5)-C(2) | 2.741 |
| C(6)-C(2) | 2.419 | C(8)-C(2) | 2.317 |
| H(41)-C(3) | 2.035 | C(5)-C(3) | 2.406 |
| C(6)-C(3) | 2.815 | C(7)-C(3) | 2.387 |
| C(4)-H(31) | 2.049 | H(41)-H(31) | 2.349 |
| H(51)-C(4) | 2.032 | C(6)-C(4) | 2.411 |
| C(7)-C(4) | 2.718 | C(5)-H(41) | 2.034 |
| H(51)-H(41) | 2.324 | H(61)-C(5) | 2.058 |
| C(7)-C(5) | 2.346 | C(6)-H(51) | 2.035 |
| H(61)-H(51) | 2.359 | C(8)-C(6) | 2.611 |
| C(7)-H(61) | 2.048 | C(9)-C(8) | 2.530 |
| H(101)-C(8) | 2.777 | C(12)-C(8) | 3.176 |
| H(101)-C(9) | 1.810 | C(11)-C(9) | 2.573 |
| H(121)-C(9) | 2.022 | H(122)-C(9) | 2.022 |
| H(123)-C(9) | 2.022 | C(10)-H(91) | 1.813 |
| C(11)-H(91) | 2.659 | C(12)-H(91) | 1.911 |
| H(121)-H(91) | 2.211 | H(122)-H(91) | 2.163 |
| H(111)-C(10) | 1.988 | H(112)-C(10) | 1.988 |
| C(12)-C(10) | 2.543 | H(121)-C(10) | 2.664 |
| C(11)-H(101) | 1.912 | H(112)-H(101) | 2.196 |
| C(12)-H(101) | 2.719 | H(112)-H(111) | 1.568 |
| H(181)-H(111) | 2.264 | H(122)-H(121) | 1.567 |
| H(123)-H(121) | 1.567 | H(123)-H(122) | 1.568 |
| H(141)-C(13) | 2.075 | H(142)-C(13) | 2.075 |
| H(143)-C(13) | 2.075 | H(151)-C(13) | 2.066 |
| H(152)-C(13) | 2.066 | H(153)-C(13) | 2.066 |
| H(161)-C(13) | 2.060 | H(162)-C(13) | 2.060 |
| H(163)-C(13) | 2.060 | C(17)-C(13) | 3.056 |
| C(23)-C(13) | 3.066 | C(15)-C(14) | 2.504 |
| H(151)-C(14) | 2.698 | H(152)-C(14) | 2.654 |
| C(16)-C(14) | 2.487 | H(162)-C(14) | 2.656 |
| H(163)-C(14) | 2.656 | H(142)-H(141) | 1.567 |
| H(143)-H(141) | 1.567 | C(15)-H(141) | 2.695 |
| H(143)-H(142) | 1.568 | C(16)-H(142) | 2.660 |
| C(15)-H(143) | 2.661 | C(16)-H(143) | 2.660 |

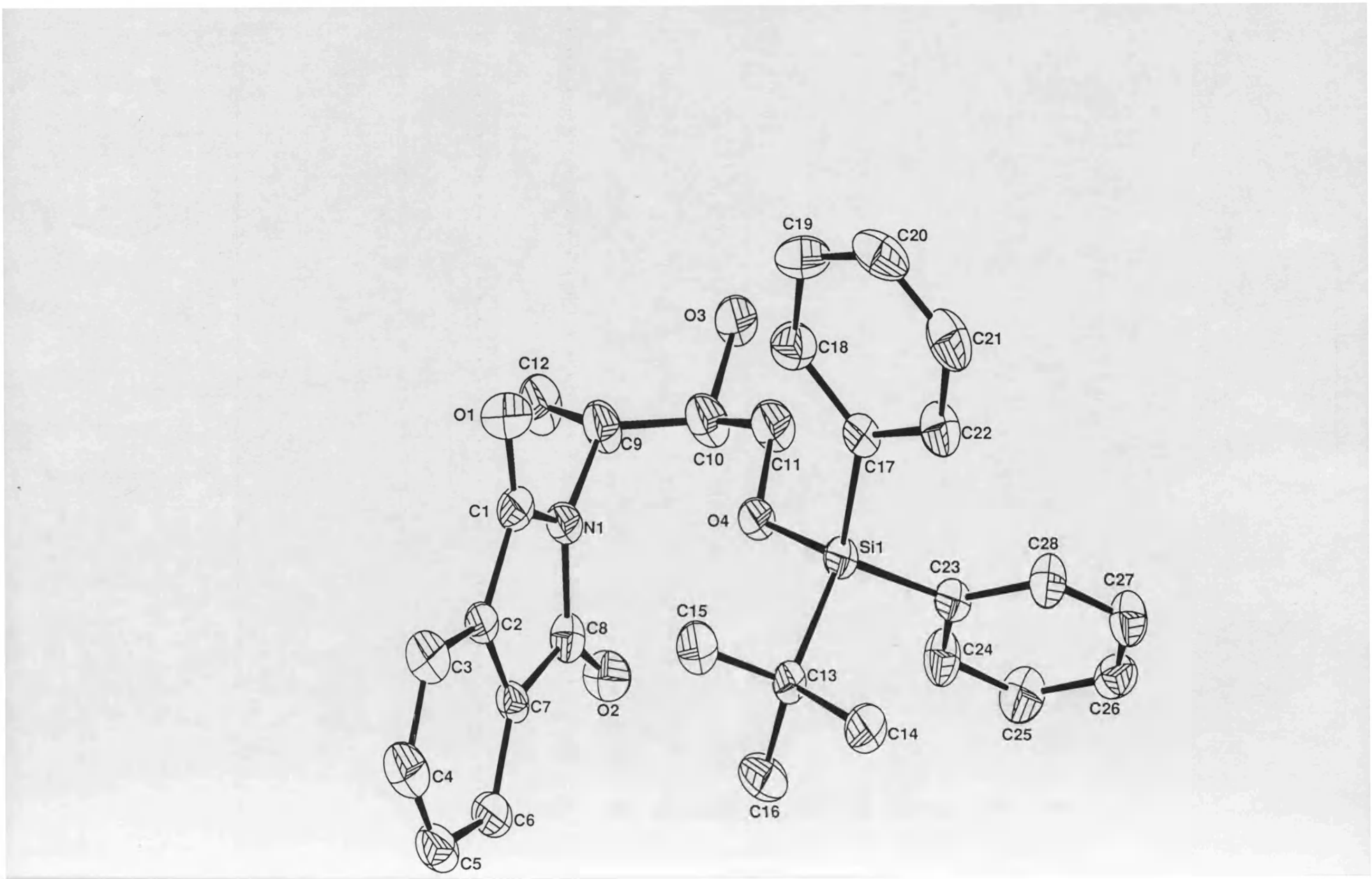
| | | | |
|---|---|---|---|
| C(16)-C(15) | 2.495 | H(161)-C(15) | 2.688 |
| H(162)-C(15) | 2.651 | H(152)-H(151) | 1.567 |
| H(153)-H(151) | 1.567 | H(153)-H(152) | 1.568 |
| C(16)-H(152) | 2.671 | C(16)-H(153) | 2.672 |
| H(162)-H(161) | 1.567 | H(163)-H(161) | 1.567 |
| H(163)-H(162) | 1.568 | H(181)-C(17) | 2.035 |
| C(19)-C(17) | 2.429 | C(20)-C(17) | 2.808 |
| C(21)-C(17) | 2.420 | H(221)-C(17) | 2.031 |
| C(23)-C(17) | 3.121 | H(191)-C(18) | 2.040 |
| C(20)-C(18) | 2.380 | C(21)-C(18) | 2.720 |
| C(22)-C(18) | 2.347 | C(19)-H(181) | 2.028 |
| H(191)-H(181) | 2.326 | H(201)-C(19) | 2.028 |
| C(21)-C(19) | 2.348 | C(22)-C(19) | 2.728 |
| C(20)-H(191) | 2.025 | H(201)-H(191) | 2.331 |
| H(211)-C(20) | 2.011 | C(22)-C(20) | 2.376 |
| C(21)-H(201) | 2.017 | H(211)-H(201) | 2.315 |
| H(221)-C(21) | 2.027 | C(22)-H(211) | 2.034 |
| H(221)-H(211) | 2.320 | H(281)-C(22) | 2.732 |
| H(281)-H(221) | 2.085 | H(241)-C(23) | 2.046 |
| C(25)-C(23) | 2.435 | C(26)-C(23) | 2.822 |
| C(27)-C(23) | 2.421 | H(281)-C(23) | 2.034 |
| H(251)-C(24) | 2.040 | C(26)-C(24) | 2.392 |
| C(27)-C(24) | 2.731 | C(28)-C(24) | 2.361 |
| C(25)-H(241) | 2.034 | H(251)-H(241) | 2.328 |
| H(261)-C(25) | 2.032 | C(27)-C(25) | 2.356 |
| C(28)-C(25) | 2.736 | C(26)-H(251) | 2.022 |
| H(261)-H(251) | 2.332 | H(271)-C(26) | 2.027 |
| C(28)-C(26) | 2.393 | C(27)-H(261) | 2.039 |
| H(271)-H(261) | 2.337 | H(281)-C(27) | 2.024 |
| C(28)-H(271) | 2.030 | H(281)-H(271) | 2.314 |

Intermolecular:

| | | | |
|---|---|---|---|
| H(201)-O(1a) | 2.618 | H(211)-O(1b) | 2.666 |
| O(3)-O(2c) | 2.855 | H(61)-O(3d) | 2.638 |
| H(61)-H(3d) | 2.221 | H(271)-H(3b) | 2.092 |

Key to symmetry operations relating designated atoms to reference atoms at (x,y,z):

(a) 1.0-x,1.0-y,1.0-z
(b) x,1.0-y,-0.5+z
(c) 0.5-x,0.5-y,1.0-z
(d) x,-1.0+y,z


C19
C20
O3
C18
C21
C12
O1
C9
C10
C11
C17
C22
C1
N1
O4
Si1
C28
C23
C27
C15
C2
C8
C24
C3
C13
C7
O2
C26
C14
C25
C4
C16
C6
C5